CoronaVac

O que a ciência comprova

fundação butantan

INSTITUTO BUTANTAN
A serviço da vida

GOVERNO DO ESTADO
DE SÃO PAULO

Sumário

1. Gera alta resposta imune

1.1. CoronaVac protege 80% contra hospitalizações e mortes em pessoas reinfectadas pelo SARS-CoV-2, mostra estudo

Um estudo de mundo real realizado no Brasil com cerca de 20 mil pessoas voltou a comprovar a efetividade da CoronaVac contra casos graves e hospitalizações por Covid-19. A análise mostrou que mais de 80% dos indivíduos reinfectados pelo SARS-CoV-2 que haviam tomado a CoronaVac se recuperaram da doença sem necessidade de hospitalização. Publicado na The Lancet Infectious Diseases, o trabalho foi feito por cientistas do Brasil e do exterior, de instituições como Universidade de São Paulo e Instituto Oswaldo Cruz (Fiocruz), além de universidades americanas como Yale e Stanford.

Entre fevereiro de 2020 e novembro de 2021, foram identificados 213 mil brasileiros (vacinados e não vacinados) que tiveram Covid-19 sintomática após o início do programa de vacinação. Para comparar a efetividade dos imunizantes CoronaVac, AstraZeneca, Pfizer e Janssen, os cientistas selecionaram 22,5 mil casos de todo o país que testaram positivo para a reinfecção pelo SARS-CoV-2, sendo que 1.545 acabaram sendo hospitalizados e 290 morreram.

Dos 22,5 mil casos analisados, 8 mil foram imunizados – 42,8% haviam tomado a CoronaVac, 40% receberam a AstraZeneca, 14,9% a Pfizer e 2,2% a Janssen. Entre os vacinados com CoronaVac, a efetividade contra hospitalização e morte foi de 81,3%. O percentual foi similar ao das vacinas AstraZeneca (89,9%) e Pfizer (89,7%), e superior aos resultados da Janssen (57,7%). Vale ressaltar que a imunização com CoronaVac no Brasil começou primeiro, tendo envolvido, em larga maioria, idosos acima de 60 anos, um público mais vulnerável ao agravamento dos casos de Covid-19.

De acordo com os pesquisadores, as análises foram concentradas em indivíduos que foram previamente infectados pelo coronavírus para responder se e até que ponto as vacinas conferem proteção adicional contra infecção sintomática e desfechos graves.

"Preocupações têm sido levantadas sobre respostas de anticorpos neutralizantes menos robustas e duráveis em indivíduos que receberam a CoronaVac em comparação com outras vacinas. Nós mostramos que a CoronaVac fornece altos níveis de proteção contra desfechos graves da doença", reforçam os cientistas no artigo.

Publicado em: 01/04/2022

Effectiveness of CoronaVac, ChAdOx1 nCoV-19, BNT162b2, and Ad26.COV2.S among individuals with previous SARS-CoV-2 infection in Brazil: a test-negative, case-control study

Thiago Cerqueira-Silva, Jason R Andrews*, Viviane S Boaventura, Otavio T Ranzani, Vinicius de Araújo Oliveira, Enny S Paixão, Juracy Bertoldo Júnior, Tales Mota Machado, Matt D T Hitchings, Murilo Dorion, Margaret L Lind, Gerson O Penna, Derek A T Cummings, Natalie E Dean, Guilherme Loureiro Werneck, Neil Pearce, Mauricio L Barreto, Albert I Ko, Julio Croda†, Manoel Barral-Netto†*

Summary

Background COVID-19 vaccines have proven highly effective among individuals without a previous SARS-CoV-2 infection, but their effectiveness in preventing symptomatic infection and severe outcomes among individuals with previous infection is less clear. We aimed to estimate the effectiveness of four COVID-19 vaccines against symptomatic infection, hospitalisation, and death for individuals with laboratory-confirmed previous SARS-CoV-2 infection.

Methods Using national COVID-19 notification, hospitalisation, and vaccination datasets from Brazil, we did a test-negative, case-control study to assess the effectiveness of four vaccines (CoronaVac [Sinovac], ChAdOx1 nCoV-19 [AstraZeneca], Ad26.COV2.S [Janssen], and BNT162b2 [Pfizer-BioNtech]) for individuals with laboratory-confirmed previous SARS-CoV-2 infection. We matched cases with RT-PCR positive, symptomatic COVID-19 with up to ten controls with negative RT-PCR tests who presented with symptomatic illnesses, restricting both groups to tests done at least 90 days after an initial infection. We used multivariable conditional logistic regression to compare the odds of test positivity and the odds of hospitalisation or death due to COVID-19, according to vaccination status and time since first or second dose of vaccines.

Findings Between Feb 24, 2020, and Nov 11, 2021, we identified 213 457 individuals who had a subsequent, symptomatic illness with RT-PCR testing done at least 90 days after their initial SARS-CoV-2 infection and after the vaccination programme started. Among these, 30 910 (14·5%) had a positive RT-PCR test consistent with reinfection, and we matched 22 566 of these cases with 145 055 negative RT-PCR tests from 68 426 individuals as controls. Among individuals with previous SARS-CoV-2 infection, vaccine effectiveness against symptomatic infection 14 or more days from vaccine series completion was 39·4% (95% CI 36·1–42·6) for CoronaVac, 56·0% (51·4–60·2) for ChAdOx1 nCoV-19, 44·0% (31·5–54·2) for Ad26.COV2.S, and 64·8% (54·9–72·4) for BNT162b2. For the two-dose vaccine series (CoronaVac, ChAdOx1 nCoV-19, and BNT162b2), effectiveness against symptomatic infection was significantly greater after the second dose than after the first dose. Effectiveness against hospitalisation or death 14 or more days from vaccine series completion was 81·3% (75·3–85·8) for CoronaVac, 89·9% (83·5–93·8) for ChAdOx1 nCoV-19, 57·7% (–2·6 to 82·5) for Ad26.COV2.S, and 89·7% (54·3–97·7) for BNT162b2.

Interpretation All four vaccines conferred additional protection against symptomatic infections and severe outcomes among individuals with previous SARS-CoV-2 infection. The provision of a full vaccine series to individuals after recovery from COVID-19 might reduce morbidity and mortality.

Funding Brazilian National Research Council, Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro, Oswaldo Cruz Foundation, JBS, Instituto de Salud Carlos III, Spanish Ministry of Science and Innovation, and Generalitat de Catalunya.

Introduction

As of March 11, 2022, over 450 million confirmed cases of COVID-19 have been reported since the start of the pandemic,[1] and the true cumulative incidence has probably been several times greater.[2] Within a year of the identification of SARS-CoV-2, multiple vaccines were developed, found to be highly efficacious among seronegative individuals in clinical trials, and introduced into national vaccination programmes.[3,4] Coverage of COVID-19 vaccination has varied across populations due to inequalities in access and public hesitancy. Additionally, public debate has emerged about the need for vaccination among people who have had a previous SARS-CoV-2 infection[5] and, if so, whether a single dose is sufficient.[6,7] The emergence of more transmissible variants with enhanced immune

Lancet Infect Dis 2022

Published Online March 31, 2022 https://doi.org/10.1016/S1473-3099(22)00140-2

See Online/Comment https://doi.org/10.1016/S1473-3099(22)00207-9

*Contributed equally

†Contributed equally

Instituto Gonçalo Moniz (T Cerqueira-Silva MD, Prof V S Boaventura MD, V de Araújo Oliveira MD, Prof A I Ko MD, Prof M Barral-Netto MD) **and Center for Data and Knowledge Integration for Health** (V de Araújo Oliveira, J Bertoldo Júnior MSc, Prof M L Barreto MD, Prof M Barral-Netto), **Fiocruz, Salvador, BA, Brazil; Faculdade de Medicina** (T Cerqueira-Silva, Prof V S Boaventura, V de Araújo Oliveira, Prof M Barral-Netto) **and Instituto de Saúde Coletiva** (J Bertoldo Júnior, Prof M L Barreto), **Universidade Federal da Bahia, Salvador, BA, Brazil; Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, CA, USA** (J R Andrews MD); **Barcelona Institute for Global Health, Barcelona, Spain** (O T Ranzani PhD); **Pulmonary Division, Heart Institute, Hospital das Clínicas, Faculdade de Medicina, São Paulo, SP, Brazil** (O T Ranzani); **Department of Infectious Disease Epidemiology** (E S Paixão PhD) **and Department of Medical Statistics** (Prof N Pearce PhD), **London School of Hygiene & Tropical Medicine, London, UK; Diretoria de Tecnologia da Informação, Universidade Federal de Ouro Preto, Ouro**

Articles

Preto, MG, Brazil (T Mota Machado MSc); **Department of Biostatistics, College of Public Health & Health Professions** (M D T Hitchings PhD), **Department of Biology** (Prof D A T Cummings PhD), **and Emerging Pathogens Institute** (Prof D A T Cummings), **University of Florida, Gainesville, FL, USA; Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, USA** (M Dorion, M L Lind PhD, Prof A I Ko, Prof J Croda PhD); **Núcleo de Medicina Tropical, Universidade de Brasília, Brasília, DF, Brazil** (G O Penna PhD); **Escola Fiocruz de Governo, Fiocruz Brasília, Brasília, DF, Brazil** (G O Penna); **Department of Biostatistics & Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, GA, USA** (N E Dean PhD); **Departamento de Epidemiologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil** (Prof G Loureiro Werneck PhD); **Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil** (Prof J Croda); **Fiocruz Mato Grosso do Sul, Fiocruz, Campo Grande, MS, Brazil** (Prof J Croda)

Correspondence to:
Prof Julio Croda, Fundação Oswaldo Cruz, Mato Grosso do Sul, Campo Grande, MS, 79081-746, Brazil
julio.croda@fiocruz.br

Research in context

Evidence before this study

We searched PubMed, medRxiv, and SSRN for articles published from Jan 1, 2020, to Feb 14, 2022, with no language restrictions, using the search terms "vaccine effectiveness" AND "previous*" AND ("SARS-CoV-2" OR "COVID-19"). We found several studies evaluating ChAdOx1 nCoV-19 (AstraZeneca) and BNT162b2 (tozinameran; Pfizer-BioNtech), and one additionally reporting on mRNA-1273 (elasomeran; Moderna) and Ad26.COV2.S (Janssen), which found that individuals who were previously infected and were vaccinated had lower risk of symptomatic SARS-CoV-2 infection than those who were unvaccinated. One study found that for individuals who were previously infected, the risk of hospitalisation was lower after a full series of BNT162b2 or mRNA-1273 than for those who were unvaccinated. One study reported on effectiveness of an inactivated virus vaccine (BBV152; Bharat Biotech International) against reinfection, and no studies reported on effectiveness of CoronaVac among individuals who were previously infected. Scarce evidence is available comparing effectiveness of one dose versus two doses of vaccine among individuals with previous infection.

Added value of this study

We used national databases of COVID-19 case surveillance, laboratory testing, and vaccination from Brazil to investigate the effectiveness of CoronaVac, ChAdOx1 nCoV-19, Ad26.COV2.S, and BNT162b2 among individuals with a previous, laboratory-confirmed SARS-CoV-2 infection. We matched more than 22 000 RT-PCR-confirmed re-infections with more than 145 000 RT-PCR-negative controls, using a test-negative design. All four vaccines were effective against symptomatic SARS-CoV-2 infection, with effectiveness from 14 days after series completion ranging from 39·4% (95% CI 36·1–42·6) for CoronaVac to 64·8% (54·9–72·4) for BNT162b2. For vaccines with two-dose regimens, the second dose provided significantly increased effectiveness compared with one dose alone. Effectiveness against COVID-19-associated hospitalisation or death from 14 days after series completion was over 80% for CoronaVac, ChAdOx1 nCoV-19, and BNT162b2.

Implications of all the available evidence

We found evidence that these four vaccines, using three different platforms, all provide protection against symptomatic SARS-CoV-2 infection and severe outcomes to individuals who were previously infected, with a second dose conferring significant additional benefits. These results support the provision of a full vaccine series among individuals with previous SARS-CoV-2 infection.

escape, and the resulting waves of infection and reinfection, have renewed questions about the importance of vaccination in individuals who have had COVID-19.[8,9]

SARS-CoV-2 infection induces robust T-cell and B-cell responses,[10] and the risk of symptomatic infection and severe outcomes is lower among people with previous SARS-CoV-2 infection than among naive individuals.[11] Emerging evidence suggests that vaccination with ChAdOx1 nCoV-19 (AstraZeneca), Ad26.COV2.S (Janssen), BNT162b2 (tozinameran; Pfizer-BioNtech), or mRNA-1273 (elasomeran; Moderna) confers additional protection against symptomatic reinfection among individuals with previous SARS-CoV-2 infection.[12–18] However, only one study has assessed protection against severe outcomes in previously infected individuals, with just 75 hospital admissions and two deaths.[18] Moreover, data for inactivated vaccines, which account for almost half of all doses given globally, are still needed.[19]

Brazil has recorded more than 22 million SARS-CoV-2 infections and 600 000 deaths as of Nov 15, 2021. On Jan 18, 2021, a national COVID-19 immunisation programme was initiated, which has used four vaccines of three different classes: inactivated virus (CoronaVac; Sinovac), viral vector (ChAdOx1 nCoV-19 and Ad26.COV2.S), and mRNA (BNT162b2). We used national disease surveillance and vaccination databases to estimate the effectiveness of these four vaccines among individuals with laboratory-confirmed previous SARS-CoV-2 infection against symptomatic infection, hospitalisation, and death.

Methods

Study design, population, and data sources

We did a test-negative, case-control study to evaluate the effectiveness of four vaccines (CoronaVac, ChAdOx1 nCoV-19, Ad26.COV2.S, and BNT162b2) in individuals with previous SARS-CoV-2 infection in Brazil. The study population included individuals with a previous positive RT-PCR or rapid antigen test for SARS-CoV-2 who presented again to health facilities with symptomatic illness and were tested for SARS-CoV-2 at least 90 days after their first positive test.[20] We matched positive tests (cases) to negative tests (controls).

We used data from several national data sources: a deterministically linked dataset comprised of the Programa Nacional de Imunizações, which contains records of all vaccines administered in Brazil; the e-SUS Notifica, which contains records of suspected and confirmed COVID-19 cases in outpatient clinics; and the Sistema de Informação da Vigilância Epidemiológica da Gripe, which contains records of severe acute respiratory illnesses, including COVID-19 hospitalisations and deaths.[21–25] All data were pseudo-anonymised with a common unique identifier provided by the Brazilian Ministry of Health. The research protocol was approved by the Brazilian National Commission in Research Ethics (4.921.308).

Brazil's national COVID-19 immunisation programme commenced on Jan 18, 2021. Rollout plans were determined at the state and local level; health-care workers and older individuals were the first groups to be eligible, with age criteria for eligibility decreasing over time. Four vaccines have been offered in immunisation programmes in Brazil: CoronaVac, provided as a two-dose series with a 4-week interval between doses; ChAdOx1 nCoV-19, provided as a two-dose series with a 12-week interval between doses that was subsequently reduced to 8 weeks in some states; Ad26.COV2.S, provided as a single dose series; and BNT162b2, provided as a two-dose series with an initial 12-week interval that was subsequently reduced to 3 weeks in some states. Brazil's national guidelines recommend that individuals who were previously infected be vaccinated 4 weeks or more after infection, and this recommendation did not change during the study period.

Eligibility and selection of cases and controls

Inclusion criteria for this study included age 18 years or older, previous SARS-CoV-2 infection confirmed by RT-PCR or rapid antigen test, and a second exam (RT-PCR test) fulfilling the following criteria: being associated with an event of acute respiratory symptomatic illness and occurring within 10 days of symptom onset, being done at least 90 days after the individual's first positive test, and occurring after the vaccination programme began in Brazil (Jan 18, 2021). We included individuals whose first infection occurred between Feb 24, 2020, and Aug 13, 2021, and with a subsequent RT-PCR test being done between Jan 18, 2021, and Nov 11, 2021.

We excluded individuals for whom data were incomplete on age, sex, location of residence, vaccination status, or testing status or dates; those who received different vaccines for their first and second dose; those whose time interval between the first and second doses was less than 14 days; and those vaccinated before the first infection or less than 14 days after the first infection. For tests, we excluded negative tests that were followed by a positive test within 7 days (to avoid misclassification of cases as controls), tests done after the second positive test, tests for which the individual's symptom onset date occurred after notification of the suspected case in the surveillance system (to exclude individuals without symptoms at the time of testing), tests done in individuals without symptoms, and tests done after a third vaccine dose, as this analysis was not powered to examine effectiveness of third doses. In some cases, more than one negative test from one individual was available for matching, and we included these as candidates for matching if they met the described eligibility criteria.

We matched cases, defined as positive SARS-CoV-2 RT-PCR tests from previously infected, symptomatic individuals, with controls, defined as negative SARS-CoV-2 RT-PCR tests from previously infected, symptomatic individuals. We did not attempt to ascertain causality between SARS-CoV-2 infection and hospitalisation or death as this information was not available. Instead, we defined hospitalisation or death related to COVID-19 using a commonly used, temporally defined surveillance case definition for COVID-19-related outcomes: a positive SARS-CoV-2 RT-PCR test accompanied by hospital admission or death occurring within 28 days of the sample collection date. For the analysis of hospitalisation or death, we selected matched sets from the overall matched dataset in which cases were positive tests from patients admitted to hospital or who died, and we fitted the model described to each subset. For severe outcomes, controls thus represented negative tests from patients in ambulatory or hospital settings who had RT-PCR testing, to reflect the population at risk for that outcome. We did not require controls for the severe outcomes analysis to be negative tests from patients admitted to hospital or who died, as the goal was to estimate overall effectiveness against severe outcomes. We matched one case to a maximum of ten controls, with replacement, by date of RT-PCR testing (±10 days), age (±5 years), sex, and municipality of residence. Individuals who were selected as cases could also serve as controls if they had negative tests that were collected more than 7 days before their positive test.

Statistical analyses

We calculated standardised differences for demographic characteristics of matched cases and controls, considering a difference higher than 0·1 for variables not included in the exact match to be significant;[26,27] for exact matched variables, no differences exist within each stratum of the analysis. The primary exposure of interest was vaccination status, which was categorised by vaccine and according to the vaccination status of the individual at the time of RT-PCR test collection as unvaccinated, 0–13 days after the first dose, 14 days or more after the first dose, 0–13 days after the second dose, or 14 days or more after the second dose. Post-second dose status is not applicable to Ad26.COV2.S. We considered vaccine effectiveness against symptomatic SARS-CoV-2 infection and against COVID-19-related hospitalisation or death among individuals with previous confirmed SARS-CoV-2 infection 14 days or more after vaccine series completion (two doses for CoronaVac, ChAdOx1 nCoV-19, and BNT162b2 and one dose for Ad26.COV2.S) to be the primary estimands of interest. We considered effectiveness in the 6 days after the first vaccine dose to be an indicator of bias, because we expected protection to be minimal during this time and substantial differences in risk could reflect residual confounding between the vaccinated and unvaccinated populations.[28]

We estimated vaccine effectiveness (1–odds ratio) using conditional logistic regression, accounting for the matched design, with vaccination status (including number of doses and time period since dose) as the

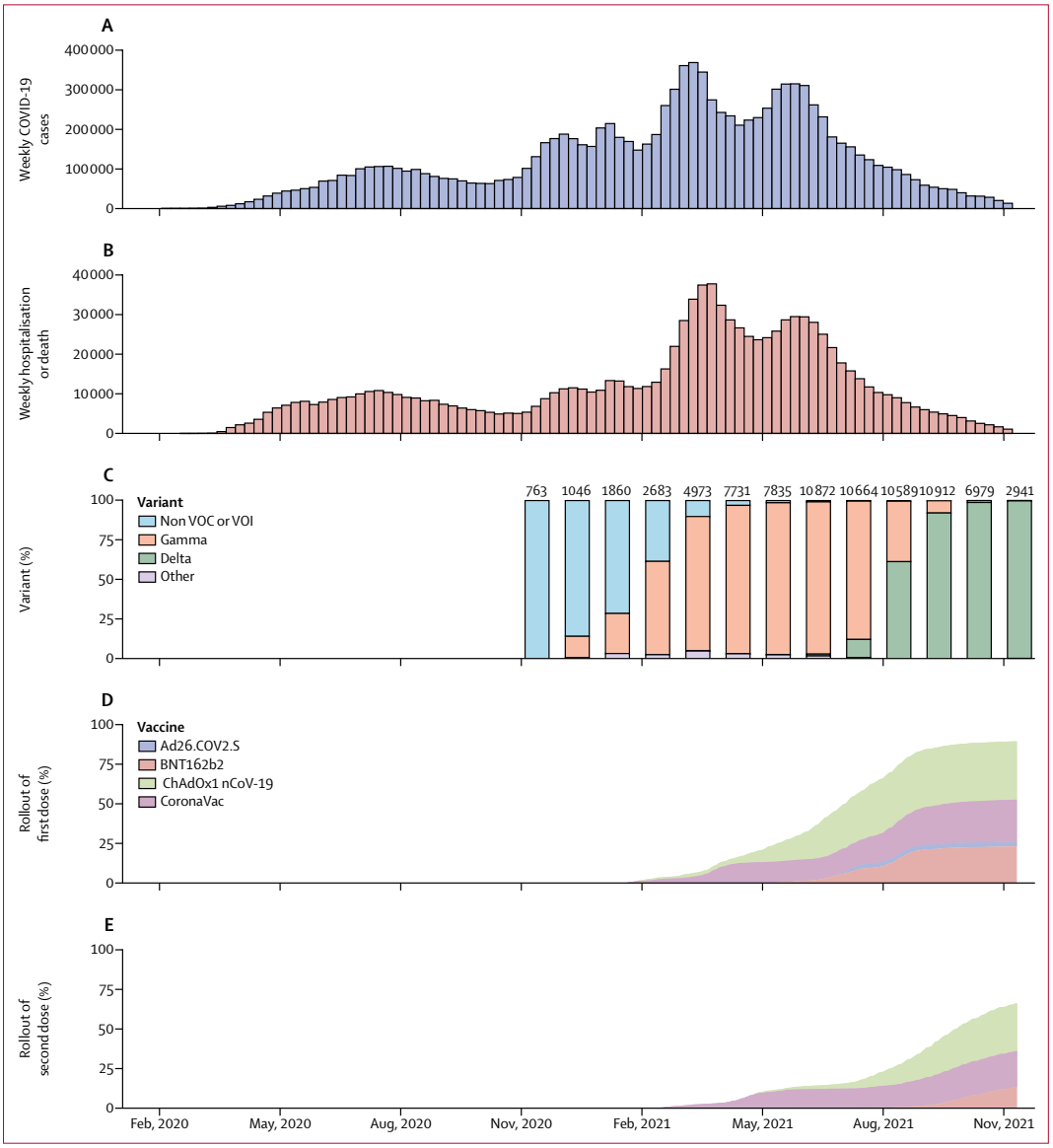


Figure 1: **Temporal trends in COVID-19 cases, hospitalisation or deaths, variants, and vaccination coverage from national databases in Brazil**
Weekly numbers of symptomatic COVID-19 cases (A); COVID-19-associated hospitalisations or deaths reported in national databases (B); monthly proportions of variants among sequenced SARS-CoV-2 samples, with the number of sequenced viruses shown above each bar (C); and cumulative proportion of the population older than 11 years who received a first (D) or second (E) dose of each vaccine. VOC=variant of concern. VOI=variant of interest.

predictor and adjusting for the number of reported chronic comorbidities (diabetes, cardiovascular disease, obesity, chronic kidney disease, and immunosupression, categorised as none, one, and at least two), pregnancy, postpartum period, self-reported race, days elapsed between the first positive test and the second test (as a restricted cubic spline), and whether the individual was admitted to hospital during their first SARS-CoV-2 infection. For severe outcomes, age (as a continuous variable) was also included due to anticipated residual confounding and observed improved model fit and Bayesian Information Criterion.

We did subgroup analyses in which we assessed vaccine effectiveness by age (18–49 years *vs* ≥50 years), time since vaccine series completion (14–90 days *vs* >90 days; to assess for possible waning), and time from

initial positive test to vaccination (91–180 days *vs* 181–613 days). We used generalised linear hypothesis tests for comparisons across different vaccination status, and the confidence intervals and p values were not adjusted for multiple comparisons. All data processing and analyses were done in R (version 4.1.1), using the packages tidyverse, multcomp, MatchIt, and survival.

Role of the funding source

Julio Croda is affiliated with Oswaldo Cruz and received support from the Oswaldo Cruz Foundation for this work. The Oswaldo Cruz Foundation and the other funders of the study did not have any further role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Brazil has had two COVID-19 epidemic waves up to the end of 2021, with the first occurring between July and September 2020, and the second between February and June 2021, during which the gamma (P.1) variant was dominant (figure 1). Brazil's national vaccination programme commenced on Jan 18, 2021; 50% of the adult population (83 million individuals) had received a first vaccine dose by July 7, 2021. Between Feb 24, 2020, and Nov 11, 2021, more than 23 million individuals had valid SARS-CoV-2 tests and 11 million were confirmed cases (figure 2). Among these, we identified 213 457 individuals who had a subsequent, symptomatic illness with RT-PCR testing done at least 90 days after their initial SARS-CoV-2 infection and after the vaccination programme commenced. Among these, 30 910 (14·5%) had a positive RT-PCR test consistent with reinfection. We matched 22 566 of these cases with 145 055 negative RT-PCR tests from 68 426 individuals as controls. Among cases, 1545 (6·8%) were admitted to hospital and 290 (1·3%) died within 28 days of a positive SARS-CoV-2 RT-PCR; 1564 (6·9%) were admitted to hospital or died (table).

Demographics and clinical characteristics of eligible and matched sets are presented in the table. The median age of the matched population was 36 years (IQR 29–44), approximately 60% of cases and controls were women, and the median time between first infection and the subsequent RT-PCR test was similar between cases (216 days, IQR 146–291) and controls (223 days, 154–295). The southeast region of Brazil, which includes São Paulo and Rio de Janeiro and is the most populous region, accounted for 49·2% of matched cases and 51·3% of controls. This was followed by the northeast region, which is the second most populous region, and then the central-west, south, and north regions (table). 39·8% of cases and 53·2% of controls resided in a state capital; due to exact matching on city, we observed no differences within each stratum of analysis.

The majority of cases (14 566 [64·5%] of 22 566) and controls (83 290 [57·4%] of 145 055) were unvaccinated at the time of the test. Among vaccinated individuals (39 717), 17 008 (42·8%) received CoronaVac, 15 897 (40·0%) received ChAdOx1 nCoV-19, 5 935 (14·9%) received BNT162b2, and 877 (2·2%) received Ad26.COV2.S. Demographic characteristics were similar among vaccine recipients included in the analysis, but recipients of ChAdOx1 nCoV-19 tended to be older (p<0·0001) and have more comorbidities (p<0·0001; appendix pp 2–3). The median time between vaccination and testing was 34 days (IQR 17–61) for individuals who received only one dose and 59 days (27–105) for individuals who received two doses, which differed by each vaccine (appendix p 12).

Effectiveness against symptomatic SARS-CoV-2 reinfection 14 days or more from vaccine series completion was 39·4% (95% CI 36·1–42·6) for CoronaVac, 56·0% (51·4–60·2) for ChAdOx1 nCoV-19, 44·0% (31·5–54·2) for Ad26.COV2.S, and 64·8% (54·9–72·4) for BNT162b2 (figure 3). The two-dose vaccines (CoronaVac, ChAdOx1

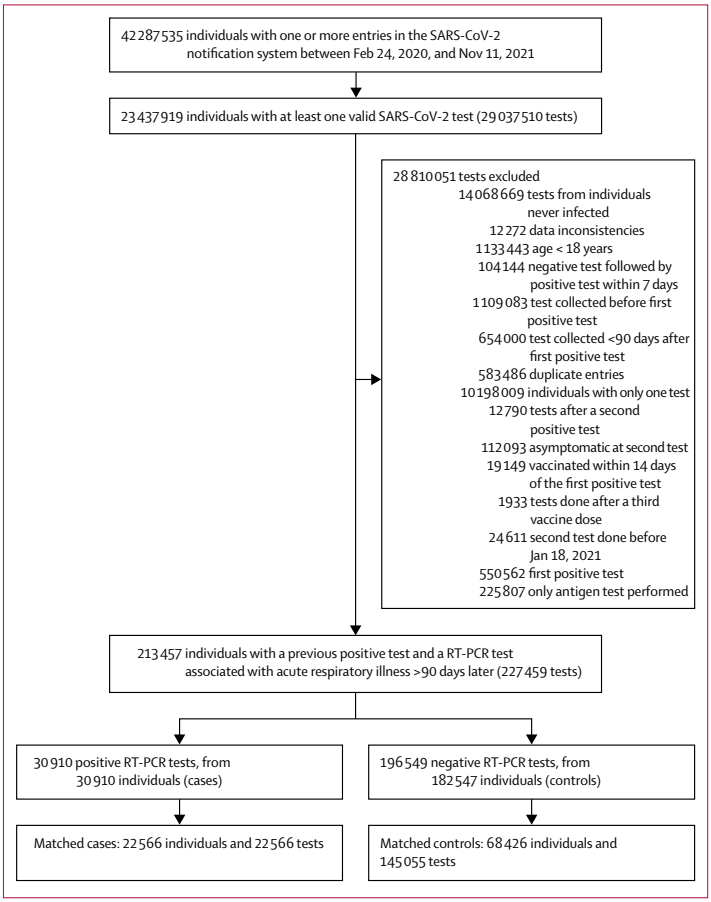


Figure 2: **Flowchart of the study population from surveillance databases and selection of matched cases and controls**
Cases and controls were matched on age (±5 years), sex, municipality, and date of test (±10 days).

See Online for appendix

	Eligible population		Matched sets		Standardised difference
	Cases	Controls	Cases	Controls	
Individuals	30 910	182 547	22 566	68 426	..
Tests	30 910	196 549	22 566	145 055	..
Age, years	38 (29–47)	37 (28–47)	37 (29–46)	36 (29–44)	0·066
Sex	..	..	..	..	0·047
Female	18 106 (58·6%)	119 134 (60·6%)	13 631 (60·4%)	90 931 (62·7%)	..
Male	12 804 (41·4%)	77 415 (39·4%)	8935 (39·6%)	54 124 (37·3%)	..
Race	..	..	..	..	0·039
White	13 841 (44·8%)	109 923 (55·9%)	10 302 (45·7%)	67 403 (46·5%)	..
Mixed	11 363 (36·8%)	53 401 (27·2%)	7998 (35·4%)	50 788 (35·0%)	..
Black	1420 (4·6%)	9034 (4·6%)	1052 (4·7%)	7572 (5·2%)	..
Indigenous or Asian	2081 (6·7%)	9305 (4·7%)	1437 (6·4%)	8751 (6·0%)	..
Missing	2205 (7·1%)	14 886 (7·6%)	1777 (7·9%)	10 541 (7·3%)	..
Region of residence	..	..	..	..	0·085
Central west	3260 (10·5%)	46 968 (23·9%)	2302 (10·2%)	12 997 (9·0%)	..
North	2406 (7·8%)	9724 (4·9%)	1870 (8·3%)	12 372 (8·5%)	..
Northeast	8268 (26·7%)	30 027 (15·3%)	5297 (23·5%)	30 489 (21·0%)	..
South	2823 (9·1%)	16 251 (8·3%)	1991 (8·8%)	14 745 (10·2%)	..
Southeast	14 153 (45·8%)	93 579 (47·6%)	11 106 (49·2%)	74 452 (51·3%)	..
Residence in state capital	9250 (29·9%)	51 128 (26·0%)	8982 (39·8%)	77 198 (53·2%)	0·271
Medical comorbidities	..	..	..	..	..
None	25 988 (84·1%)	166 655 (84·8%)	19 271 (85·4%)	124 964 (86·1%)	0·027
One	3552 (11·5%)	22 178 (11·3%)	2459 (10·9%)	15 360 (10·6%)	..
Two or more	1370 (4·4%)	7716 (3·9%)	836 (3·7%)	4731 (3·3%)	..
Days from first positive test to second test	210 (144–285)	217 (154–293)	216 (146–291)	223 (154–295)	0·060
Hospitalised during first infection	1220 (3·9%)	9481 (4·8%)	781 (3·5%)	6507 (4·5%)	0·052
Hospitalisation (up to 28 days)	2508 (8·1%)	3770 (1·9%)	1545 (6·8%)	2196 (1·5%)	..
Death (up to 28 days)	559 (1·8%)	663 (0·3%)	290 (1·3%)	386 (0·3%)	..
Hospitalisation or death	2554 (8·3%)	3829 (1·9%)	1564 (6·9%)	2238 (1·5%)	..

Data are n, n (%), or median (IQR). Percentages were calculated using number of tests as the denominator. Matching was based on tests rather than individuals, with up to ten controls matched, with replacement, per case.

Table: **Characteristics, vaccination status, and outcomes of individuals eligible for and matched into case-control sets**

nCoV-19, and BNT162b2) all showed a significant increase in protection from 14 days or more after the first dose to 14 days or more after the second dose. For CoronaVac, effectiveness was twice as high in the period of 14 days or more after the second dose compared with that in 14 days or more after the first (p<0·0001). Only CoronaVac showed protection (21·0%, 2·3–36·1) against symptomatic infection within 6 days of the first dose, which we used as a test of bias (appendix p 4).

From 14 days after completion of the vaccine series, effectiveness against COVID-19-related hospitalisation or death was 81·3% (75·3–85·8) for CoronaVac, 89·9% (83·5–93·8) for ChAdOx1 nCoV-19, 57·7% (–2·6 to 82·5) for Ad26.COV2.S, and 89·7% (54·3–97·7) for BNT162b2 (figure 4). Effectiveness 14 days or more after a single dose was lowest for CoronaVac (35·3%, 7·9–54·5). Effectiveness against hospitalisation or death was significantly greater 14 days or more after two doses than 14 days or more after one dose for CoronaVac (p<0·0001) and ChAdOx1 nCoV-19 (p<0·0001), whereas for BNT162b2, the increase was not significant (p=0·091). We found no evidence of protection for all four vaccines against COVID-19-related hospitalisation or death within 6 days of the first dose (appendix p 4).

For the primary estimands of vaccine effectiveness against symptomatic SARS-CoV-2 infection and against COVID-19-related hospitalisation or death 14 days or more after vaccine series completion, we found no differences between age groups (≥50 years *vs* 18–49 years; appendix p 5). For three of the vaccines, we saw a non-significant increase in effectiveness against symptomatic infection for vaccination given more than 180 days after previous infection compared with 91–180 days, whereas we observed a significant increase

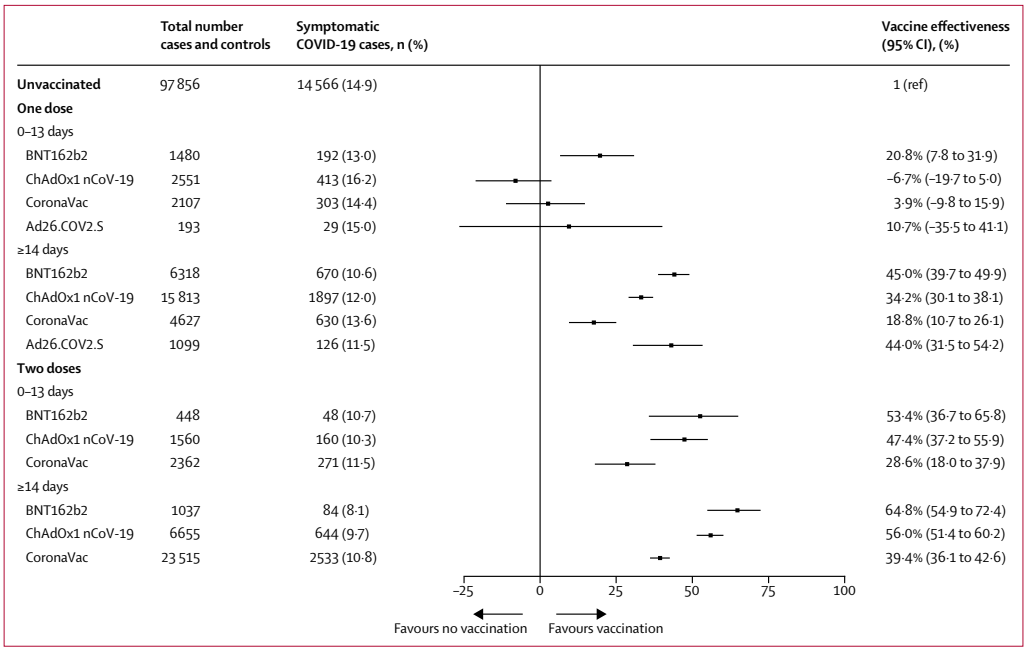


Figure 3: **Effectiveness of BNT162b2, ChAdOx1 nCoV-19, CoronaVac, and Ad26.COV2.S vaccines against symptomatic COVID-19 among individuals with previous SARS-CoV-2 infection**

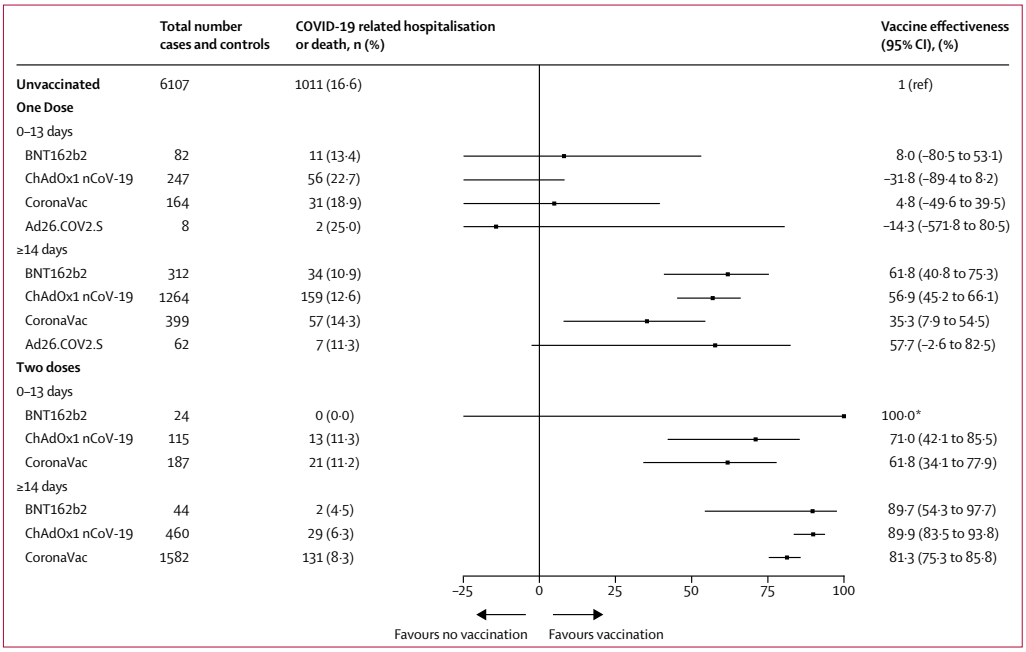


Figure 4: **Effectiveness of BNT162b2, ChAdOx1 nCoV-19, CoronaVac, and Ad26.COV2.S vaccines against COVID-19-associated hospitalisation or death among individuals with previous SARS-CoV-2 infection**

*95% CI could not be estimated owing to zero events in this group.

for BNT162b2 (35·3% *vs* 70·7%, p=0·011; appendix p 5). We found no differences in effectiveness against symptomatic infection when comparing the periods of 14–90 days and more than 90 days after vaccine series completion. For hospitalisation and death, effectiveness of ChAdOx1 nCoV-19 was greater at more than 90 days after completion compared with that at 14–90 days (95·1% *vs* 86·6%; p=0·007), whereas effectiveness was lower for CoronaVac at more than 90 days than at 14–90 days (74·4% *vs* 86·6%, p=0·012; appendix p 5).

Discussion

In this nationwide, population-based study among individuals with confirmed previous SARS-CoV-2 infection, we observed a high degree of additional protection of four vaccines against symptomatic COVID-19 and severe outcomes. For the three vaccines with two doses (CoronaVac, ChAdOx1 nCoV-19, and BNT162b2), additional protection against symptomatic infection was observed after the second dose, reaching 39% to 65%, and protection against hospitalisation or death exceeded 80% 14 days or more after the second dose. These results support vaccination, including the full vaccine series, among individuals with previous SARS-CoV-2 infection.

Public debate has occurred about whether individuals who were previously infected need to be vaccinated, due to substantial immunity conferred by SARS-CoV-2 infection.[5] Additionally, in view of data showing robust immune responses after a first vaccine dose in individuals who were previously infected, some have argued that two doses are not necessary.[6,7] Indeed, several countries recommend that a single vaccine dose is sufficient for individuals who were previously infected.[29–31] We found that a second dose of CoronaVac, ChAdOx1 nCoV-19, and BNT162b2 provided significant additional protection against symptomatic infections and severe disease. A recent study has shown that IgG antibodies to the receptor binding domain in individuals who recovered from COVID-19 declined to about 35% of their individual level by 9 months.[32] Additionally, repeated antigen exposures were observed to increase antibody diversity, which might improve protection against emergent variants.[32] Taken together, these findings might help explain the additional benefits of a second vaccine dose among individuals who were previously infected, despite robust immune responses to the first dose.[33]

The results of this analysis are consistent with studies reporting that individuals with previous SARS-CoV-2 infection who received ChAdOx1 nCoV-19 and BNT162b2 had a lower risk of symptomatic COVID-19 than those who were previously infected and unvaccinated.[12,13,15,16] Direct comparison with vaccine effectiveness estimates from these studies is challenged by differences in design, with most studies reporting risk in comparison with individuals who were unvaccinated and without a previous SARS-CoV-2 infection. However, inferred protection from those studies ranged from 40% to 94%, consistent with the magnitude of protection against symptomatic infection found for ChAdOx1 nCoV-19 (56·0%) and BNT162b2 (64·8%) in this study. Our analysis also adds new estimates on effectiveness of the CoronaVac and Ad26.COV2.S vaccines among individuals who were previously infected, finding that these vaccines provide more modest levels of protection against symptomatic infection, consistent with their lower effectiveness in naive populations.[21,34] Concerns have been raised about less robust and durable neutralising antibody responses in individuals naive to SARS-CoV-2 who have received CoronaVac compared with other vaccines.[35] We found that two doses of CoronaVac provided high levels of protection against severe outcomes (81·3%, 95% CI 75·3–85·8). As CoronaVac is among the most widely used vaccines in the world, these findings have broad implications for many national programmes.[19]

To our knowledge, only one previous study reported vaccine effectiveness against COVID-19-related hospitalisation or death among individuals who were previously infected; with just 75 outcomes and three vaccines evaluated, the power of that study was limited for assessing vaccine and dose-specific effectiveness, but estimates ranged from 58% (BNT162b2) to 68% (mRNA-1273), with no significant protection from Ad26.COV2.S.[18] We found that protection against these severe outcomes, from 14 days after the second dose, was greater than 80% for the three two-dose vaccines (CoronaVac, ChAdOx1 nCoV-19, and BNT162b2). These results are consistent with recent data showing that individuals who were previously infected have even greater increases in T-cell and B-cell responses after vaccination than those without previous infection.[36] This high degree of hybrid immunity, from infections and vaccination, might explain why Brazil, despite having similar vaccination coverage as the USA and many European countries, did not have a similar increase in hospitalisations and deaths in the period in which the delta (B.1.617.2) variant become dominant.

Effectiveness against severe outcomes was lower (57·7%) for the single-dose Ad26.COV2.S vaccine than for the vaccines given in two-dose series, although the confidence limits were wide. The Ad26.COV2.S vaccine was used in a more focal rollout from June to July, 2021, and far fewer individuals received this vaccine compared with the others, such that we had modest power to characterise the effectiveness of this vaccine against severe outcomes. Brazil's Ministry of Health now recommends that individuals who received this vaccine receive a second dose after 60 days.

We focused our analyses on individuals who were previously infected to address the question of whether and to what extent vaccines confer additional protection against symptomatic infection and severe outcomes. We did not compare against individuals without a previous infection because their risk of exposure might be different, which could lead to biased estimates in this population-based study. Additionally, the misclassification

of individuals who were previously infected as not having been previously infected is a substantial risk, due to incomplete surveillance and asymptomatic infections; restricting vaccine effectiveness analysis to individuals with PCR-confirmed previous infection avoids this bias. Although much discussion has occurred concerning the relative protection conferred by infection-derived and vaccine-derived immunity, from a medical and public health standpoint, the crucial question is understanding whether individuals with previous infection would benefit from vaccination. This study suggests that individuals infected before vaccination benefit from strong protection against severe outcomes with all four vaccines studied.

A major difficulty with observational studies of vaccine effectiveness is the risk of confounding, whereby differences in the vaccinated and unvaccinated populations are associated with the risk of a COVID-19 diagnosis. The matched, test-negative design has been recommended by WHO to mitigate risk of confounding introduced by care-seeking and diagnostic access; nevertheless, residual confounding might occur. We used vaccine effectiveness in the 6 days after the first dose as a bias indicator, in that differences during this period before vaccine-conferred protection is expected could indicate confounding.[28] We only observed significant effectiveness in this time interval for one vaccine (CoronaVac) and one outcome (symptomatic infection); over the 7–13-day time window, no effectiveness for this vaccine was observed (appendix p 4). Whether the effectiveness observed over days 0–6 reflects bias or chance among the eight bias indicator tests (4 vaccines with 2 outcomes each) is unclear, but the absence of effects in the 7–13-day window might point away from systematic differences in recipients of CoronaVac regarding SARS-CoV-2 risk. For BNT162b2, we found modest protection in the 7–13-day window (appendix p 4). In clinical trials of BNT162b2, efficacy was apparent from approximately 11 days after the first dose.[3] Given the rapid and robust immune responses after first vaccination among individuals who were previously infected, we believe these findings are consistent with early vaccine-conferred immunity.

This study has several limitations. First, we were not powered to assess vaccine effectiveness by age groups. We compared effectiveness in individuals older and younger than 50 years and did not observe major differences. The mean age of our study population was 36 years, with 75% younger than 45 years; these findings might not generalise to older populations. Second, there were differences in the timing of introduction and eligibility for each of the vaccines. This should prompt some caution in the comparison of effectiveness between vaccines, as the calendar period and median duration from second dose differed somewhat between vaccines. For example, if effectiveness wanes over time, vaccines used earlier would have lower effectiveness than those introduced later. Additionally, changes in variant distribution during the study period could alter effectiveness by time since vaccination. We did not have individual-level data on variants, which precluded assessment of variant-specific vaccine effectiveness. Different types and collection methods for RT-PCR tests are used throughout the country, which might have varying accuracy, and specific information about these characteristics are not recorded in the national databases. We used a matched, test-negative design with multivariable regression to reduce non-vaccine-related differences between cases and controls; however, unmeasured differences could exist that lead to confounding.[37] In particular, there were differences in the allocation of specific vaccines that might have been associated with unmeasured risk of COVID-19 or severe outcomes, which should prompt caution in the comparison of vaccine effectiveness between vaccines. This study included individuals who presented to health facilities and underwent diagnostic testing who might differ from individuals who did not seek medical care and might not be generalisable to that population. Finally, our study was unable to address the important question of when vaccines should be given to individuals with previous SARS-CoV-2 infection. To avoid misclassification of reinfections, we only considered tests done at least 90 days after the initial infection.

The accelerated development of effective vaccines against COVID-19 has been a remarkable scientific achievement but, as of March 11, 2022, 37·4% of the world's population has yet to receive a first dose, and a substantial proportion of these individuals have already been infected with SARS-CoV-2.[1] The results of this study provide evidence for the benefits of vaccination among individuals who have already been infected with SARS-CoV-2, with all four studied vaccines conferring substantial reductions in hospitalisation and death due to COVID-19. Ensuring vaccine access to individuals with previous infection might be particularly important amid reports of the omicron (B.1.1.529) variant, which suggest that immunity conferred by previous infection is reduced.[9,10,38] The expanded, equitable rollout of vaccines for all individuals remains crucial for mitigating the continued threat posed by SARS-CoV-2.

Contributors

JRA, JC, and MB-N conceived the idea for the study. All authors contributed to the study design. TC-S, JRA, and OTR developed the statistical analysis plan and wrote the code for statistical analyses. TC-S, VdAO, JBJ, and MB-N had access to the raw data, and TC-S and MB-N verified the underlying data. TC-S, MB-N, VdAO, and MLB organised the data linkage. All authors contributed to interpretation of the study findings. JRA and TC-S drafted the manuscript. All authors critically revised the manuscript and approved the final version for submission.

Declaration of interests

MB-N reports grants from the Fazer o Bem Faz Bem programme from JBS. AIK reports grants from Bristol Myers Squibb, Regeneron, and Serimmune; and grants and personal fees from Tata Medical Devices, outside the submitted work. VdAO, VSB, MLB, JC, and MB-N are employees of Fiocruz, a federal public institution, which manufactures Vaxzevria (ChAdOx1 nCoV-19 vaccine) in Brazil through a full technology

transfer agreement with AstraZeneca. Fiocruz allocates all its manufactured products to the Ministry of Health for public health use. All other authors declare no competing interests.

Data sharing

One of the study coordinators (MB-N) signed a term of responsibility on using each database made available by the Brazilian Ministry of Health. Each member of the research team signed a term of confidentiality before accessing the data. Data were manipulated in a secure computing environment, ensuring protection against data leakage. The Brazilian National Commission in Research Ethics approved the research protocol (CONEP approval number 4.921.308). Our agreement with the Ministry of Health for accessing the databases patently denies authorisation of access to a third party. Any request for assessing the databases must be addressed to the Brazilian Ministry of Health. We used anonymised secondary data following the Brazilian Personal Data Protection General Law, but they are vulnerable to re-identification by third parties as they contain dates of relevant health events regarding the same person. To protect the research participants' privacy, the approved research protocol authorises only the dissemination of aggregated data, such as the ones presented here.

Acknowledgments

This study was partly supported by a donation from the Fazer o Bem Faz Bem programme from JBS. GLW, MLB, JC, and MB-N are research fellows from the Brazilian National Research Council. GLW acknowledges the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (E-26/210.180/2020). JC is supported by the Oswaldo Cruz Foundation (Edital COVID-19—*resposta rápida* 48111668950485). OTR is funded by a Sara Borrell fellowship (CD19/00110) from the Instituto de Salud Carlos III. OTR acknowledges support from the Spanish Ministry of Science and Innovation through the Centro de Excelencia Severo Ochoa 2019–2023 programme and from the Generalitat de Catalunya through the Centres de Recerca de Catalunya programme.

References

1 WHO. WHO coronavirus (COVID-19) dashboard. 2021. https://covid19.who.int/ (accessed March 11, 2022).

2 Chen X, Chen Z, Azman AS, et al. Serological evidence of human infection with SARS-CoV-2: a systematic review and meta-analysis. *Lancet Glob Health* 2021; **9:** e598–609.

3 Thomas SJ, Moreira ED Jr, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine through 6 months. *N Engl J Med* 2021; **385:** 1761–73.

4 Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021; **397:** 99–111.

5 Block J. Vaccinating people who have had COVID-19: why doesn't natural immunity count in the US? *BMJ* 2021; **374:** n2101.

6 Dolgin E. Is one vaccine dose enough if you've had COVID? What the science says. *Nature* 2021; **595:** 161–62.

7 Frieman M, Harris AD, Herati RS, et al. SARS-CoV-2 vaccines for all but a single dose for COVID-19 survivors. *EBioMedicine* 2021; **68:** 103401.

8 Cele S, Jackson L, Khoury DS, et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. *Nature* 2021; published online Dec 23. https://doi.org/10.1038/s41586-021-04387-1.

9 Pulliam JRC, van Schalkwyk C, Govender N, et al. Increased risk of SARS-CoV-2 reinfection associated with emergence of the omicron variant in South Africa. *medRxiv* 2021; published online Dec 2. https://doi.org/10.1101/2021.11.11.21266068 (preprint).

10 Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 2021; **371:** eabf4063.

11 Abu-Raddad LJ, Chemaitelly H, Bertollini R. Severity of SARS-CoV-2 reinfections as compared with primary infections. *N Engl J Med* 2021; **385:** 2487–89.

12 Cavanaugh AM, Spicer KB, Thoroughman D, Glick C, Winter K. Reduced risk of reinfection with SARS-CoV-2 after COVID-19 vaccination—Kentucky, May–June 2021. *MMWR Morb Mortal Wkly Rep* 2021; **70:** 1081–83.

13 Gazit S, Shlezinger R, Perez G, et al. Comparing SARS-CoV-2 natural immunity to vaccine-induced immunity: reinfections versus breakthrough infections. *medRxiv* 2021; published online Aug 25. https://doi.org/10.1101/2021.08.24.21262415 (preprint).

14 Abu-Raddad LJ, Chemaitelly H, Ayoub HH, et al. Association of prior SARS-CoV-2 infection with risk of breakthrough infection following mRNA vaccination in Qatar. *JAMA* 2021; **326:** 1930–39.

15 Hall V, Foulkes S, Insalata F, et al. Effectiveness and durability of protection against future SARS-CoV-2 infection conferred by COVID-19 vaccination and previous infection; findings from the UK SIREN prospective cohort study of healthcare workers March 2020 to September 2021. *medRxiv* 2021; published online Dec 1. http://www.medrxiv.org/content/10.1101/2021.11.29.21267006v1 (preprint).

16 Pouwels KB, Pritchard E, Matthews PC, et al. Effect of delta variant on viral burden and vaccine effectiveness against new SARS-CoV-2 infections in the UK. *Nat Med* 2021; **27:** 2127–35.

17 Monge S, Olmedo C, Alejos B, Lapeña MF, Sierra MJ, Limia A. Direct and indirect effectiveness of mRNA vaccination against severe acute respiratory syndrome coronavirus 2 in long-term care facilities, Spain. *Emerg Infect Dis* 2021; **27:** 2595–603.

18 Levin-Rector A, Firestein L, McGibbon E, et al. Reduced odds of SARS-CoV-2 reinfection after vaccination among New York City adults, June–August 2021. *medRxiv* 2021; published online Dec 11. http://www.medrxiv.org/content/10.1101/2021.12.09.21267203v1 (preprint).

19 Mallapaty S. China's COVID vaccines have been crucial—now immunity is waning. *Nature* 2021; **598:** 398–99.

20 WHO. Enhancing response to omicron SARS-CoV-2 variant: technical brief and priority actions for member states. 2022. https://www.who.int/docs/default-source/coronaviruse/2022-01-07-global-technical-brief-and-priority-action-on-omicron---corr2.pdf?sfvrsn=918b09d_23&download=true (accessed Jan 18, 2022).

21 Ranzani OT, Hitchings MDT, Dorion M, et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of COVID-19 in Brazil: test negative case-control study. *BMJ* 2021; **374:** n2015.

22 Ranzani OT, Bastos LSL, Gelli JGM, et al. Characterisation of the first 250 000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. *Lancet Respir Med* 2021; **9:** 407–18.

23 Oliveira EA, Colosimo EA, Simões E Silva AC, et al. Clinical characteristics and risk factors for death among hospitalised children and adolescents with COVID-19 in Brazil: an analysis of a nationwide database. *Lancet Child Adolesc Health* 2021; **5:** 559–68.

24 Cerqueira-Silva T, Oliveira VA, Boaventura VS, et al. Influence of age on the effectiveness and duration of protection of Vaxzevria and CoronaVac vaccines: a population-based study. *Lancet Reg Health Am* 2022; **6:** 100154.

25 Katikireddi SV, Cerqueira-Silva T, Vasileiou E, et al. Two-dose ChAdOx1 nCoV-19 vaccine protection against COVID-19 hospital admissions and deaths over time: a retrospective, population-based cohort study in Scotland and Brazil. *Lancet* 2022; **399:** 25–35.

26 Flury BK, Riedwyl H. Standard distance in univariate and multivariate analysis. *Am Stat* 1986; **40:** 249–51.

27 Austin PC. Using the standardized difference to compare the prevalence of a binary variable between two groups in observational research. *Commun Stat Simul Comput* 2009; **38:** 1228–34.

28 Hitchings MDT, Lewnard JA, Dean NE, et al. Use of recently vaccinated individuals to detect bias in test-negative case-control studies of COVID-19 vaccine effectiveness. *medRxiv* 2021; published online July 2. https://doi.org/10.1101/2021.06.23.21259415 (preprint).

29 Federal Office of Public Health. Switzerland. Coronavirus: vaccination. 2021. https://www.bag.admin.ch/bag/en/home/krankheiten/ausbrueche-epidemien-pandemien/aktuelle-ausbrueche-epidemien/novel-cov/impfen.html#-995735508 (accessed Dec 16, 2021).

30 German Federal Ministry of Health. Proof of vaccination as defined in the COVID-19 protective measures exemption directive and the directive on coronavirus entry regulations. 2021. https://www.pei.de/EN/newsroom/dossier/coronavirus/coronavirus-content.html?cms_pos=3 (accessed Dec 16, 2021).

31 Haute Autorité de Santé. Stratégie de vaccination contre le SARS-CoV-2—vaccination des personnes ayant un antécédent de COVID-19. 2021. https://www.has-sante.fr/jcms/p_3237271/en/strategie-de-vaccination-contre-le-sars-cov-2-vaccination-des-personnes-ayant-un-antecedent-de-covid-19 (accessed Dec 16, 2021).

32 Li C, Yu D, Wu X, et al. Twelve-month specific IgG response to SARS-CoV-2 receptor-binding domain among COVID-19 convalescent plasma donors in Wuhan. *Nat Commun* 2021; **12:** 4144.

33 Muecksch F, Weisblum Y, Barnes CO, et al. Affinity maturation of SARS-CoV-2 neutralizing antibodies confers potency, breadth, and resilience to viral escape mutations. *Immunity* 2021; **54:** 1853–68.e7.

34 Zheng C, Shao W, Chen X, Zhang B, Wang G, Zhang W. Real-world effectiveness of COVID-19 vaccines: a literature review and meta-analysis. *Int J Infect Dis* 2022; **114:** 252–60.

35 Lim WW, Mak L, Leung GM, Cowling BJ, Peiris M. Comparative immunogenicity of mRNA and inactivated vaccines against COVID-19. *Lancet Microbe* 2021; **2:** e423.

36 Angyal A, Longet S, Moore SC, et al. T-cell and antibody responses to first BNT162b2 vaccine dose in previously infected and SARS-CoV-2-naive UK health-care workers: a multicentre prospective cohort study. *Lancet Microbe* 2021; **3:** e21–31.

37 Lewnard JA, Patel MM, Jewell NP, et al. Theoretical framework for retrospective studies of the effectiveness of SARS-CoV-2 vaccines. *Epidemiology* 2021; **32:** 508–17.

38 Andrews N, Stowe J, Kirsebom F, et al. COVID-19 vaccine effectiveness against the omicron (B.1.1.529) variant. *N Engl J Med* 2022; published online March 2. https://doi.org/10.1056/NEJMoa2119451.

1.2. CoronaVac multiplica o nível de anticorpos neutralizantes em pessoas recuperadas da Covid-19, mostra estudo do Chile

Um estudo chileno publicado na revista eBioMedicine, da The Lancet Discovery Science, mostrou que a CoronaVac aumenta mais de três vezes, para acima de 2.000, os títulos médios geométricos (GMT) de anticorpos neutralizantes em indivíduos com infecção prévia de SARS-CoV-2. A pesquisa foi conduzida pela Pontifícia Universidade Católica do Chile e pela Fundação Ciência e Vida, entre outras instituições.

Em indivíduos que tiveram doença leve a moderada, a CoronaVac elevou o nível de anticorpos neutralizantes de 174 para 2057,3 GMT. Já naqueles que haviam sido hospitalizados com doença grave, e por isso apresentavam mais anticorpos para combater a infecção, o aumento foi de 700,8 para 2113,6 GMT.

Para conduzir essa análise, os cientistas avaliaram a resposta imune natural de 74 indivíduos em recuperação da Covid-19 e observaram que houve uma rápida queda de anticorpos neutralizantes (nAb) ao longo de 12 meses. Destes participantes, 30 foram posteriormente vacinados com a CoronaVac e a maioria apresentou aumento na titulação de nAb, mostrando que a vacina é eficaz na ativação das células B de memória, produtoras de anticorpos.

Além disso, a efetividade de vida real da CoronaVac no Chile foi maior do que a eficácia estimada no estudo. Enquanto a proteção estimada contra infecções por Covid-19 foi de 50%, a proteção real foi de 65,9%. A vacina também protegeu 87,5% contra hospitalizações, 90,3% contra internações em Unidades de Terapia Intensiva (UTI) e 86,3% contra mortes. "A imunidade conferida pela CoronaVac está provavelmente relacionada à indução de anticorpos neutralizantes e de mecanismos adicionais, como células T e memória imunológica", afirmam os autores no artigo.

Segundo os pesquisadores, a queda de anticorpos neutralizantes ao longo do tempo em indivíduos convalescentes reforça que a vacinação é necessária para potencializar a resposta de anticorpos e de células de memória. "Estratégias de dose de reforço também são necessárias para controlar a pandemia e evitar reinfecções com novas variantes", apontam.

Coronavac aumenta proteção contra variantes

Uma pesquisa da China publicada recentemente também mostrou que a CoronaVac potencializa a imunidade de indivíduos previamente infectados, induzindo alta atividade neutralizante contra as variantes delta, alfa e beta do vírus SARS-CoV-2, além de aumentar a quantidade de anticorpos. Os anticorpos neutralizantes contra a cepa original e contra as variantes aumentaram de sete a 17 vezes.

Publicado em: 30/03/2022

Induction of SARS-CoV-2 neutralizing antibodies by CoronaVac and BNT162b2 vaccines in naïve and previously infected individuals

Nicolás A. Muena,[a,1] Tamara García-Salum,[b,c,1] Catalina Pardo-Roa,[b,c] María José Avendaño,[b] Eileen F. Serrano,[b] Jorge Levican,[b] Leonardo I. Almonacid,[b] Gonzalo Valenzuela,[b,c] Estefany Poblete,[b] Shirin Strohmeier,[d] Erick Salinas,[b,c] Andres Muñoz,[b] Denise Haslwanter,[e] Maria Eugenia Dieterle,[e] Rohit K. Jangra,[e,f] Kartik Chandran,[e] Claudia González,[c,g] Arnoldo Riquelme,[c,h,i] Florian Krammer,[e,j] Nicole D. Tischler,[a,k,**] and Rafael A. Medina [b,c,e,*]

[a]Laboratorio de Virología Molecular, Fundación Ciencia and Vida, Santiago, Chile
[b]Department of Pediatric Infectious Diseases and Immunology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile
[c]Advanced Interdisciplinary Rehabilitation Register (AIRR) – COVID-19 Working Group, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile
[d]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[e]Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY, USA
[f]Department of Microbiology and Immunology, Louisiana State University Health Science Center-Shreveport, Shreveport, LA, USA
[g]Department of Otorhinolaryngology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile
[h]Department of Gastroenterology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile
[i]Department of Health Sciences, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile
[j]Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[k]Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, Chile

Summary

Background A major challenge of the SARS-CoV-2 pandemic is to better define "protective thresholds" to guide the global response. We aimed to characterize the longitudinal dynamics of the antibody responses in naturally infected individuals in Chile and compared them to humoral responses induced after immunization with CoronaVac-based on an inactivated whole virus -or the BNT162b2- based on mRNA-vaccines. We also contrasted them with the respective effectiveness and efficacy data available for both vaccines.

Methods We determined and compared the longitudinal neutralizing (nAb) and anti-nucleocapsid (anti-N) antibody responses of 74 COVID-19 individuals (37 outpatient and 37 hospitalized) during the acute disease and convalescence. We also assessed the antibody boosting of 36 of these individuals who were immunized after convalescence with either the CoronaVac (n = 30) or the BNT162b2 (n = 6) vaccines. Antibody titres were also measured for 50 naÿve individuals immunized with two doses of CoronaVac (n = 35) or BNT162b2 (n = 15) vaccines. The neutralizing level after vaccination was compared to those of convalescent individuals and the predicted efficacy was estimated.

Findings SARS-CoV-2 infection induced robust nAb and anti-N antibody responses lasting >9 months, but showing a rapid nAb decay. After convalescence, nAb titres were significantly boosted by vaccination with CoronaVac or BNT162b2. In naÿve individuals, the calculated mean titre induced by two doses of CoronaVac or BNT162b2 was 0·2 times and 5.2 times, respectively, that of convalescent individuals, which has been proposed as threshold of protection. CoronaVac induced no or only modest anti-N antibody responses. Using two proposed logistic models, the predicted efficacy of BNT162b2 was estimated at 97%, in close agreement with phase 3 efficacy studies, while for CoronaVac it was ~50% corresponding to the lowest range of clinical trials and below the real-life data from Chile (from February 2 through May 1, 2021 during the predominant circulation of the Gamma variant), where the estimated vaccine effectiveness to prevent COVID-19 was 62·8–64·6%.

eBioMedicine 2022;78: 103972
Published online xxx
https://doi.org/10.1016/j.ebiom.2022.103972

*Corresponding author at: Department of Pediatric Infectious Diseases and Immunology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile.
**Corresponding author at: Laboratorio de Virología Molecular, Fundación Ciencia & Vida, Av. Zañartu 1482, Santiago, Chile
E-mail addresses: ntischler@cienciavida.org (N.D. Tischler), rmedinai@uc.cl (R.A. Medina).

[1] These authors contributed equally to this work.

Articles

Interpretation The decay of nAbs titres in previously infected individuals over time indicates that vaccination is needed to boost humoral memory responses. Immunization of naÿve individuals with two doses of CoronaVac induced nAbs titres that were significantly lower to that of convalescent patients, and similar to vaccination with one dose of BTN162b2. The real life effectiveness for CoronaVac in Chile was higher than estimated; indicating that lower titres and additional cellular immune responses induced by CoronaVac might afford protection in a highly immunized population. Nevertheless, the lower nAb titre induced by two doses of CoronaVac as compared to the BTN162b2 vaccine in naÿve individuals, highlights the need of booster immunizations over time to maintain protective levels of antibody, particularly with the emergence of new SARS-CoV-2 variants.

Funding FONDECYT 1161971, 1212023, 1181799, FONDECYT Postdoctorado 3190706 and 3190648, ANID Becas/Doctorado Nacional 21212258, PIA ACT 1408, CONICYT REDES180170, Centro Ciencia & Vida, FB210008, Financiamiento Basal para Centros Científicos y Tecnológicos de Excelencia grants from the Agencia Nacional de Investigación y Desarrollo (ANID) of Chile; NIH-NIAD grants U19AI135972, R01AI132633 and contracts HHSN272201400008C and 75N93019C00051; the JPB Foundation, the Open Philanthropy Project grant 2020-215611 (5384); and by anonymous donors. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Keywords: COVID-19; Serological response; Neutralizing antibody persistence; SARS-CoV-2 vaccines; Vaccination boost

Research in context

Evidence before this study

The duration of immune protection against SARS-CoV-2 by natural infection or vaccination remains to be elucidated during the current pandemic. As a central parameter of protection, the titre of circulating neutralizing antibodies has been characterized and compared with the efficacy and effectiveness of vaccines to protect from symptomatic disease. We searched in the PubMed database for articles published up to July 26th 2021, using the terms "SARS-CoV-2" or "COVID-19" and "neutralizing antibodies", "long-lasting response", "CoronaVac vaccine" or "BNT162b2 vaccine" to identify articles related with antibody decay over time after natural infection and initial antibody titres upon vaccination. There was data available on spike-specific antibody up to 11 months after onset of symptoms. Numerous data was also available on mRNA vaccine studies, however; little independent data was available on the inactivated virus based CoronaVac vaccine. Of note, the assays for measuring neutralization varied widely and to express data as ratio of convalescence sera, the time of convalescence since the onset of symptoms was not standardized either.

Added value of this study

This study provides a direct comparison of longitudinal convalescent nAb titres after SARS-CoV-2 natural infection and those of individuals immunized with two different vaccine formulations, CoronaVac and BNT162b2. Based on the maximal response curves to SARS-CoV-2 infection we compared the mean titre of nAb response using different time frames and used them as fold comparison with titres found in naïve immunized individuals. The data was further contrasted with the estimated real-life vaccine effectiveness and efficacy to prevent COVID-19, available for these vaccines.

Implications of all the available evidence

Understanding the "threshold" of neutralizing antibody titres that confer protection against symptomatic COVID-19 would help in the management of the pandemic. This is of particular importance because of the decay of antibody levels observed over time after natural infection and vaccination, and due to the emergence of SARS-CoV-2 variants. In this study we showed that two doses CoronaVac immunization leads to initial neutralizing antibody titres that are significantly lower than that of convalescent patients and equivalent to one dose of BNT162b2. However, the real life effectiveness for CoronaVac in Chile was higher than estimated from current logistic models. Hence, further studies are required to assess if lower titres or additional cellular immune responses, might contribute to effective protection in a population with high vaccine coverage.

Introduction

The durability of circulating neutralizing antibody (nAb) responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection or vaccination has become a central question during the current pandemic to determine correlates of immune protection against disease. While the antibody dynamics during

the acute phase have been described, many studies vary considerably in the methods used.[1] Increasing evidence suggest that infected individuals can mount long-term SARS-CoV-2 spike-specific nAbs that can remain detectable for up to 11 months.[2–5] However, a "threshold" of nAb titres related with protective activity remains to be defined.[6] This definition is of particular importance where vaccine doses are sparse and for less studied vaccines that are being used widely in middle- and low-income countries.

As of June 2021, the World Health Organization (WHO) has authorized the emergency use of six vaccines, which are now also considered for distribution through the coronavirus disease 2019 (COVID-19) Vaccines Global Access (COVAX) program (https://www.who.int/initiatives/act-accelerator/covax). Limited information is currently available on the longevity of the humoral response after vaccination[7] or natural infection, and whether a vaccination boost is required for previously infected individuals, including when this should be recommended, particularly in the context of new variants of concern.[8–10] Of the authorized vaccines, limited data is available on the induction of nAbs by the inactivated virus CoronaVac vaccine (Sinovac Life Sciences Co., LTD, Beijing, China), which has been used widely in over 50 countries in the developing world, such as Brazil, Chile, Indonesia and Turkey, with a reported efficacy in protection against symptomatic COVID-19 ranging from 50 to 84%.[11] In general, there is limited information on the correlates of protection and the relationship between nAbs levels and the efficacy against symptomatic SARS-CoV-2 infection when immunized with any of the available vaccines.[12,13] To provide a framework to implement improved global vaccination strategies, it is imperative to establish correlates of protection that are evaluated and compared simultaneously across different vaccine formulations and dose schedules.[14] Hence, additional longitudinal data are needed to characterize the medium- and long-term nAb dynamics, as well as the CD4+ T cells and CD8+ T immune response[15] and the Fc- effector functions,[16] starting from the acute phase of disease of patients with mild and moderate/severe outcome. It is also important to determine and compare their memory responses upon immunization with the different vaccines currently in use (e.g. inactivated versus mRNA vaccines).

In this study we aimed to analyse the longitudinal neutralizing and anti-nucleocapsid (anti-N) antibody responses after natural infection in convalescent COVID-19 individuals, including analyses of the temporal induction and decay dynamics of these humoral responses. Using these data as a framework, we then compared these titres to those of naÿve individuals vaccinated with the CoronaVac vaccine or the BNT162b2 vaccine based on spike protein-encoding messenger RNA (BioNTech/Pfizer), which we then used to contrast them with the respective effectiveness and efficacy data available for both vaccines.

Methods

Study population and clinical metadata

The individuals included in the study are part of the CHILE COVID-19 cohort, which was established in late February of 2020, as part of a CEIRS Cross-Centre project funded by the NIH-NIAID, to study the natural history of SARS-CoV-2 in the Southern Hemisphere (Supplementary Figure 1). Of a total of 168 participants (n = 81 outpatients and n = 87 hospitalized), 74 individuals with a confirmed diagnosis for SARS-CoV-2 infection were recruited prospectively between March 5 and October 22, 2020, and were selected for longitudinal convalescent serology analyses if they had 2 or more samples during 12 months since onset of symptoms. Given that convalescent samples were obtained prior to the appearance of virus variants, in this study we assessed antibody titres against the Wuhan-like virus strain. Due to the rapid vaccination campaign in Chile, 36 of these 74 participants were immunized with 1 or 2 doses of either the CoronaVac or BNT162b2 vaccines during the follow up period within 127–398 days (4.2–13.3 months) since onset of symptoms (Supplementary Figure 2 and Supplementary Table 1). Hence, they were re-consented and followed up for an additional time period (31–126 days). Extensive metadata is collected at each visit and samples are clearly identified as being part of the convalescent period or post-vaccination period. No samples taken after vaccination were included in the longitudinal (persistent) analyses (Figures 1 and 2). The post-vaccination samples are only included in (Figures 3 and 4). We also enrolled healthy individuals (n = 50) who were recruited as controls and received two doses of the CoronaVac (n = n = 35; Sinovac Life Sciences Co., LTD, Beijing, China) or BNT162b2 (n = 15; Pfizer Manufacturing Belgium NV, Puurs, Belgium) vaccines at time intervals of 28 or 21 days, respectively. The analysis were performed considering two major groups of individuals, hospitalized and outpatients: Hospitalized individuals (n = 37) were either severe patients (n = 14), defined as those who developed pneumonia with one of the following three conditions: (1) acute respiratory failure that required invasive mechanical ventilation or a high-flow nasal cannula (HFNC) with prone position, (2) septic shock or (3) multiple organ dysfunction; moderate cases (n = 23) consisted of inpatients with pneumonia without these conditions. Outpatients (n = 37) were individuals that had mild symptoms of COVID-19 but did not meet the criteria mentioned above. Peripheral blood samples, nasopharyngeal swabs and sputum samples were collected between 2

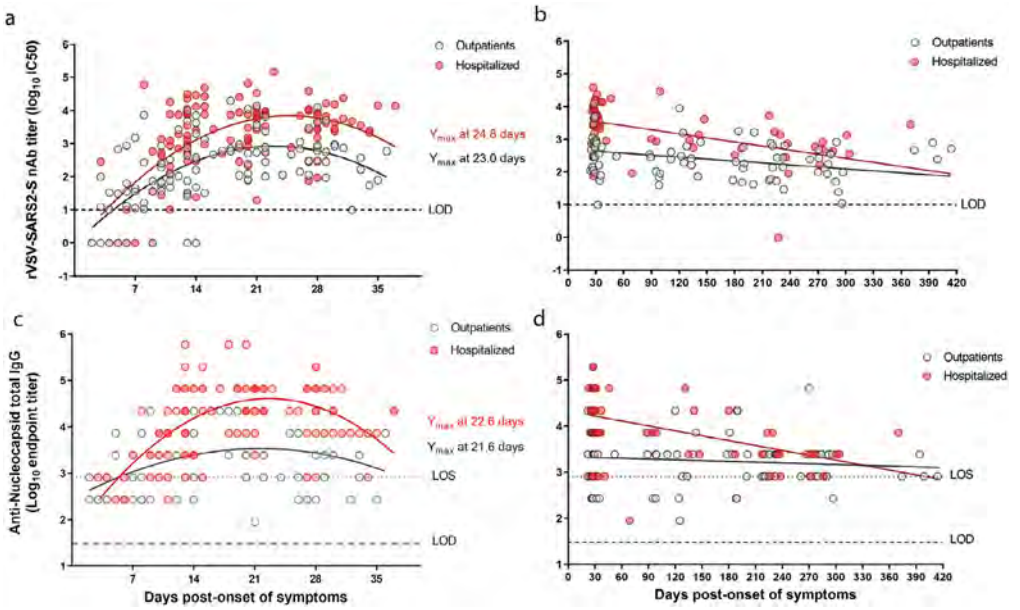


Figure 1. Longitudinal dynamics of neutralizing and anti-N antibody responses to SARS-CoV-2 infection from outpatient and hospitalized individuals. **a,b**. The half-maximum inhibitory concentration (IC50) of sera was determined by microneutralization assay of recombinant vesicular stomatitis virus carrying SARS-CoV-2 spike protein (rVSV-SARS2-S). **a**. Neutralizing antibody (nAb) titres (log10 IC50) from *n* = 30 outpatients (116 samples; grey circles) and *n* = 35 hospitalized (112 samples; red circles) at 2 to 37 days post-symptom onset. **c**. Longitudinal nAb titres (log10 IC50) from *n* = 36 outpatients (85 samples) and *n* = 31 hospitalized (58 samples) taken from day 23 (outpatients) or day 25 (hospitalized) until day 414 post-symptom onset. **c,d**. The end-point titres of anti-N IgG were determined by ELISA using a recombinant SARS-CoV-2 nucleocapsid protein. Samples and time points are the same as those in A and B. **a-c**. The second order polynomial (quadratic) curve fitting was used to establish the days at which peak titres occurred (Ymax). **b**–**d**. Continuous decay fit is shown with the red and gray line for the corresponding patient group. Every data point represents results from two technical replicates.

and 437 days after the onset of symptoms. For naÿve individuals, samples were collected 1–2 days prior to vaccination and between 10 and 30 days after the first dose but prior to the second dose and 6–31 days after the second dose. For previously infected individuals, samples were collected at time intervals corresponding to weeks 1, 2, 3, 4, and months 3, 6, 9 and 12–14 months after onset of symptoms as shown in Figs. 1 and 2. Demographic data for all patients and controls, obtained through a clinical questionnaire, are shown in Table 1.

For comparing seroconversion titres and correlates of protection, we used the same approach of Khoury et al.[12] considered as a robust approach to associate nAbs and protection. Hence, took in to account the time ranges of seven vaccine studies (e.g. of the mRNA-1273, NVX-CoV2373, BNT162b2, rAd26-S +rAd5-S, ChAdOx1 nCoV-19, Ad26.COV2.S and CoronaVac vaccines) for determining neutralization titres. This time range was 10–60 days, or not specified, from which the neutralization and protection model was developed in the Khoury et al study. We also used our own data (Figure 1a,b) that showed that some individuals have high levels of nAb during week 1 (Figure 1b). We performed initial analyses considering convalescent titres obtained in our study using time ranges of 10–37, 14–28 and 14–21 days, which showed no significant differences (Supplementary Figure 3). With this context and for broad comparisons, we adopted a more dogmatic approach and used neutralizing data from 14 to 28 days post onset of symptoms as the period at which robust nAbs are generated upon natural infection.

Plasma and serum collection

Peripheral blood was collected in both plasma separating (EDTA/purple top) and serum separating (red top) tubes and was processed by centrifugation at 2000 × g for 5 min. Limited volume of plasma and serum samples were aliquoted and stored at −80 °C. Serum samples were heated at 56 °C for 1 h before use to eliminate the risk of any potential residual virus.

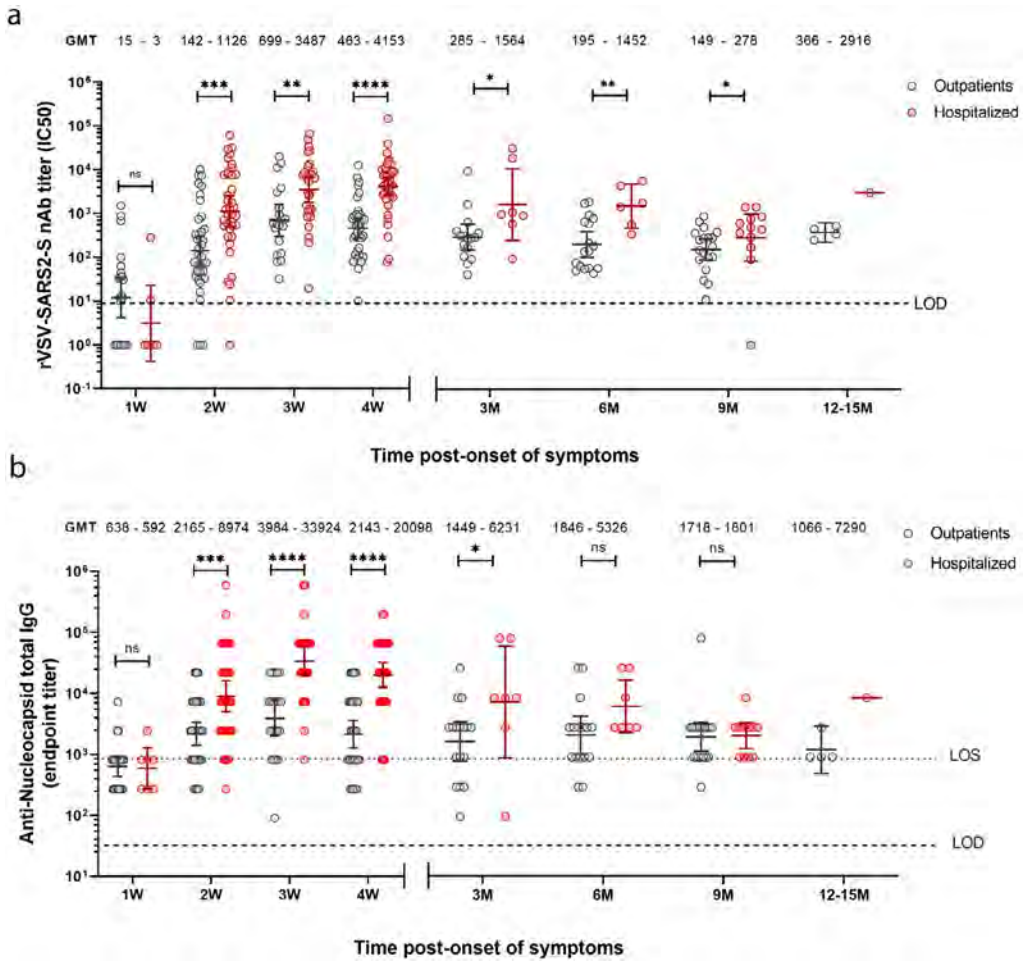


Figure 2. Comparison of neutralizing and anti-N antibody responses after SARS-CoV-2 infection of outpatient and hospitalized individuals over a 12 months period**. a.** nAb IC50 titres were determined by microneutralization assay of recombinant vesicular stomatitis virus carrying SARS-CoV-2 spike protein (rVSV-SARS2-S). **b.** End-point titres of anti-N IgG were determined by ELISA using a recombinant SARS-CoV-2 nucleocapsid protein. **a-b**. Samples were obtained for *n* = 37 outpatients (172 samples; grey circles) and *n* = 37 hospitalized (139 samples; red circles) grouped by weeks (W) or months (M) post-symptom onset (serum samples from: 1W = 1–7 days; 2W = 8–14 days; 3W = 15–21 days; 4W = 22–45 days; 3M = 46–135 days; 6M = 136–225 days; 9M = 226–315 days and 12-14M = 316–414 days). The bars indicate geometric mean titres (GMT) with 95% confidence intervals. GMTs are indicated above each data set. Dashed line represents the limit of detection (LOD) of each assay. Statistical analyses shown at the indicated time points were performed between nAb titres of outpatient and hospitalized using the unpaired two-tailed Mann-Whitney test (*$P < 0{\cdot}05$; **$P < 0{\cdot}01$; **$P < 0{\cdot}001$; ****$P < 0{\cdot}0001$; ns, non-significant). Every data point represents results from two technical replicates.

SARS-CoV-2 spike and nucleocapsid ELISAs

Overnight, 96-well plates (Immulon 4 HBX; Thermo Fisher Scientific #3355) were coated at 4 °C with 50 μL per well of a 2 μg/mL solution recombinant SARS-CoV-2 spike or nucleocapsid (GenScript #Z03488) proteins, as previously described.[3,17,18] The next morning, the plates were blocked with 3% non-fat milk prepared in PBS with 0.1% Tween 20 (PBST) for 1 h. Serial dilutions of serum and antibody samples previously inactivated by heating at 56 °C for 1 h, were diluted starting 1:50 for spike and 1:30 for nucleocapsid SARS-CoV-2 proteins were prepared and 100 μL of each dilution was added to the plates for 2 h at room temperature. For primary antibody detection a 1:3,000 dilution of goat anti-

Articles

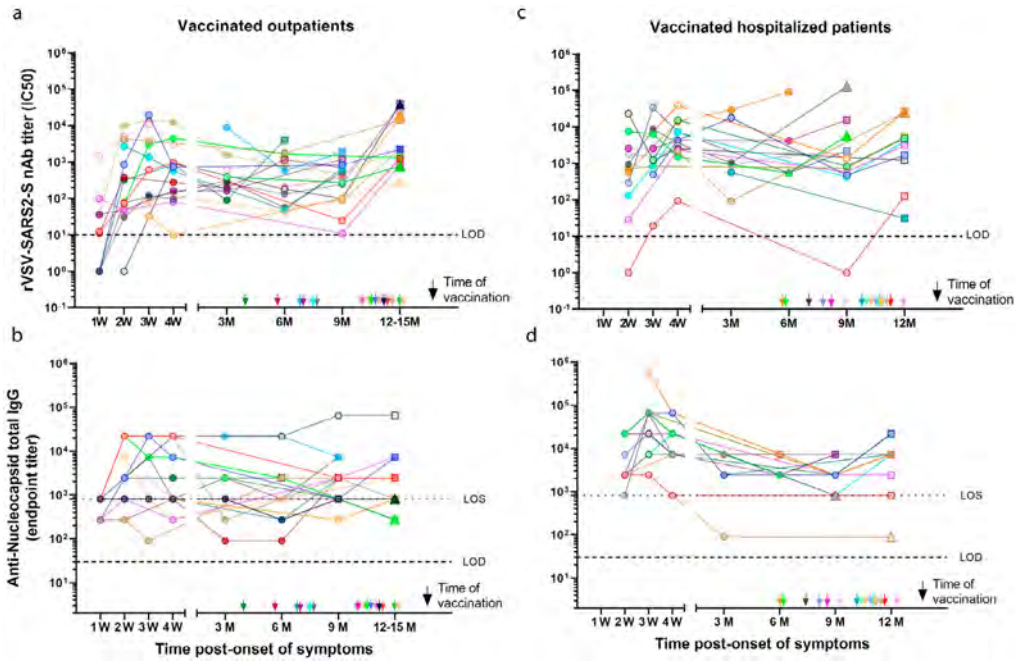


Figure 3. Longitudinal neutralizing and anti-N antibody titres to SARS-CoV-2 in previously infected before and after CoronaVac or BNT162b2 vaccination. nAb titres (IC50) obtained using a rVSV-SARS2-S microneutralization assay and end-point titres of anti-N IgG were determined by ELISA using a recombinant SARS-CoV-2 nucleocapsid protein for vaccinated previously infected outpatients (**a-b**; 20 participants) or vaccinated hospitalized patients (**c-d**; 16 participants) at different time points grouped by weeks (W) or months (M) post-symptom onset (serum samples from: 1W = 1-7 days; 2W = 8-14 days; 3W = 15-21 days; 4W = 22-45 days; 3M = 46-135 days; 6M = 136-225 days; 9M = 226-315 days and 12M = 316-405 days/12-15M = 316-495). The arrows indicate time of vaccination post-onset of symptoms (see Supplementary Table 1 for specific days of vaccination and sample collections). Circles, non-vaccinated; squares, vaccinated with CoronaVac; triangles, vaccinated with BNT162b2. Conv: convalescent; Vacc: vaccine; 0: indicates pre-vaccination samples; 1: first dose; 2: second dose. Dashed line indicates the limit of detection (LOD) of the microneutralization assay. Every data point represents results from two technical replicates.

human IgG–horseradish peroxidase (HRP) conjugated secondary antibody (Thermo Fisher Scientific # SA1-36011, RRID:AB_1075961) was added to each well for 1 h and SIGMAFAST OPD (o-phenylenediamine dihydrochloride; Sigma–Aldrich #P9187) was used as substrate. After 10 min the reaction was stopped by the addition 3 M hydrochloric acid and the optical density at 490 nm (OD490) was measured using a Synergy 4 (BioTek) plate reader. In some cases, end-point titres were calculated, with the end-point titre being the last dilution before reactivity dropped below an OD490 of <0.11. CR3022, a human monoclonal antibody reactive to the RBD of both SARS-CoV-1 and SARS-CoV-2,[19,20] was used as control. Negative and positive controls were used to standardize each assay and normalize across experiments. The limit of detection (LOD) was defined as 1:50 for spike and 1:30 for nucleocapsid. Limit of sensitivity (LOS) for the nucleocapsid assay was established on the basis of the maximal serum reactivity of uninfected subjects using samples from 16 pre-pandemic donors never exposed to SARS-CoV-2. All data represent results from two technical replicates.

SARS-CoV-2 microneutralization assay

This assay was performed as previously described.[21] Briefly, Vero E6 cells (ATCC #CRL-1586, RRID: CVCL_0574) were seeded at a density of 20,000 cells per well in a 96-well cell culture plate in complete Dulbecco's Modified Eagle Medium (cDMEM, Gibco Thermo Fisher Scientific #11995040). The following day, heat-inactivated serum samples (dilution of 1:10) were serially diluted threefold and 80 μL of each serum dilution were mixed with 80 μL of the authentic SARS-CoV-2 (USA-WA1/2020; GenBank: #MT020880) diluted to a concentration of 100 TCID50 (50% tissue culture infectious dose) and then added to a 96-well cell culture plate and allowed to incubate for 1 h at room temperature. After removing the cell culture media, the Vero E6 cells were incubated with 120 μL of the virus-

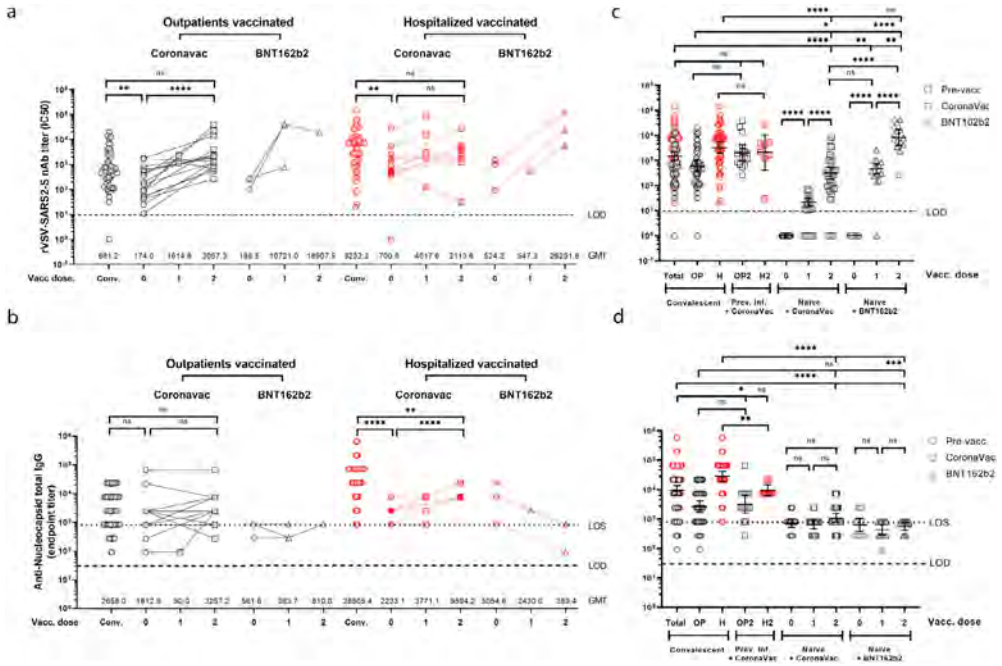


Figure 4. Neutralizing and anti-N antibody titres to SARS-CoV-2 in previously infected and naïve individuals before and after CoronaVac or BNT162b2 vaccination. nAb (**b**) and anti-N IgG (**c**) titres from 20 outpatient (42 samples) or 16 hospitalized (33 samples) individuals immunized with one or two doses of CoronaVac (30 participants) or one or two doses of BNT162b2 (6 participants) vaccines. nAb (**b**) and anti-N IgG (**d**) titres from naïve individuals after the first and second dose of CoronaVac (35 participants) or BNT162b2 (15 participants) vaccines, compared to nAb titres from convalescent patients (samples taken between days 10 and 28 from 28 outpatients (49 samples) and 34 hospitalized (58 samples) participants) and previously infected individuals (31 participants) before (31 samples) or after receiving two doses (25 samples) of the CoronaVac vaccine. Black lines represent the geometric mean titres (**c**) or end-point titres (**d**) and bars show the 95% confidence intervals. Statistics were performed using unpaired two-tailed Mann-Whitney test ((*$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$; ****$P < 0{\cdot}0001$; ns, non-significant), excluding non-seroconverted data determined as outliers. Circles, non-vaccinated; squares, vaccinated with CoronaVac; triangles, vaccinated with BNT162b2. Conv: convalescent; Vacc: vaccine; 0: indicates pre-vaccination samples; 1: first dose; 2: second dose. Dashed line indicates the limit of detection (LOD) of the microneutralization assay and dotted line represents the limit of sensitivity (LOS) of ELISA. Every data point represents results from two technical replicates.

serum mixture at 37 °C for 1 h. The virus-serum mixture was then removed from the cells and 100 μL of each corresponding serum dilution and 100 μL of 1 × MEM containing 1% fetal bovine serum (FBS, Corning # 35-010-CV) was added to the cells. After 48 h at 37 °C, the cells were fixed with 10% paraformaldehyde (Polysciences # 04018-1) for 24 h at 4 °C, permeabilized with PBS containing 0·1% Triton X-100 (Sigma-Aldrich # X100) and the plates were and blocked with 3% milk (American Bio # AB10109010000) in PBST. For detecting viral infection, a primary mAb 1C7 (anti-SARS nucleoprotein antibody generated in-house) was used at a 1:1,000 dilution and subsequently detected with a 1:3,000 dilution of a goat anti-mouse IgG−HRP (Rockland #KCB002, RRID:AB_10703407), and incubation with SIGMA-FAST OPD (Sigma-Aldrich) as described above. A cut-off value of the average of the optical density values of blank wells plus three standard deviations established for each plate was used to calculate the microneutralization titre. Microneutralization assays were performed in a facility with a biosafety level of 3 at the Icahn School of Medicine at Mount Sinai. Each data point represents results obtained from two technical replicates.

rVSV SARS-CoV-2 spike protein (rVSV-SARS2-S) microneutralization assay

To determine the nAb titres of patient sera, we used a previously described the replication-competent recombinant vesicular stomatitis virus carrying the SARS-COV-2 spike protein and coding for an enhanced green fluorescent protein (eGFP).[22] This recombinant virus has been shown to correlate well when compared to neutralization of convalescent serum with the authentic SARS-

	Outpatients (*n* = 37)	Hospitalized (*n* = 37)	P value (Outpatients vs. Hospitalized)	CoronaVac (*n* = 35)	BNT162b2 (*n* = 15)	P value (CoronaVac vs. BNT162b2)	Vaccinated previously infected (*n* = 36)	Vaccinated naïve participant (*n* = 50)	p value (previously infected vs. naïve)
Characteristics									
Male, n (%)	17 (45.9)	25 (67.6)	0.0998	11 (31.4)	3 (20)	0.5067	14 (38.9)	14 (28)	0.3531
Age, mean (range)	37 (14-66)	51 (16-83)	**0.0004**	36 (21-80)	34 (15-53)	0.6981	44 (17-83)	35 (15-80)	**0.0308**
>60 years, n (%)	5 (13.5)	12 (32.4)	0.0956	1 (2.9)	0	>0.9999	8 (22.2)	1 (2)	**0.0025**
Symptoms									
Respiratory									
Cough, n (%)	27 (73)	31 (83.8)	0.3975	NA	NA	NA	30 (83.3)	NA	NA
Dyspnea, n (%)	6 (16.2)	19 (51.4)	**0.0028**	NA	NA	NA	14 (38.9)	NA	NA
Odynophagia, n (%)	21 (56.8)	6 (16.2)	**0.0006**	NA	NA	NA	13 (36.1)	NA	NA
Chest discomfort, n (%)	3 (8.1)	5 (13.5)	0.7106	NA	NA	NA	4 (11.1)	NA	NA
Constitutional									
Fever, n (%)	22 (59.5)	31 (83.8)	**0.0377**	NA	NA	NA	26 (72.2)	NA	NA
Headache, n (%)	32 (86.5)	14 (37.8)	< **0.0001**	NA	NA	NA	23 (63.9)	NA	NA
Myalgia, n (%)	25 (67.6)	18 (48.6)	0.157	NA	NA	NA	20 (55.6)	NA	NA
Severe fatigue, n (%)	0	20 (54.1)	< **0.0001**	NA	NA	NA	11 (30.6)	NA	NA
Altered mental status, n (%)	0	3 (8.1)	0.2397	NA	NA	NA	1 (2.8)	NA	NA
Gastrointestinal									
Diarrhea, n (%)	12 (32.4)	10 (27)	0.7997	NA	NA	NA	12 (33.3)	NA	NA
Nausea/Vomiting, n (%)	6 (16.2)	9 (24.3)	0.5642	NA	NA	NA	8 (22.2)	NA	NA
Sensorial									
Ageusia, n (%)	18 (48.6)	5 (13.5)	**0.0022**	NA	NA	NA	14 (38.9)	NA	NA
Anosmia, n (%)	24 (64.9)	8 (21.6)	**0.0004**	NA	NA	NA	18 (50)	NA	NA
Comorbidities or conditions									
Obesity (BMI ≥ 30), n (%)	5 (13.5)	14 (37.8)	**0.0317**	5 (14.3)	4 (26.7)	0.4234	10 (27.8)	9 (18)	0.3038
Hypertension, n (%)	3 (8.1)	13 (35.1)	**0.0095**	3 (8.6)	2 (13.3)	0.6293	9 (25)	5 (10)	0.0797
Metabolic conditions*, n (%)	4 (10.8)	12 (32.4)	**0.0459**	2 (5.7)	1 (6.7)	>0.9999	9 (25)	3 (6)	**0.0239**
Hyperlipidemia, n (%)	4 (10.8)	7 (18.9)	0.5151	1 (2.9)	2 (13.3)	0.2107	7 (19.4)	3 (6)	0.0865
Cardiovascular disease, n (%)	0	3 (8.1)	0.2397	0	0	NA	1 (2.8)	0	0.4186
Chronic pulmonary disease, n (%)	4 (10.8)	3 (8.1)	1	0	0	NA	4 (11.1)	0	**0.0277**
Asthma, n (%)	6 (16.2)	2 (5.4)	0.2611	4 (11.4)	4 (26.7)	0.2195	5 (13.9)	8 (16)	>0.9999
Rheumatologic disease, n (%)	0	3 (8.1)	0.2397	0	1 (6.7)	0.3	1 (2.8)	1 (2)	>0.9999
Immunocompromised, n (%)	0	5 (13.5)	0.0541	0	1 (6.7)	0.3	2 (5.6)	1 (2)	0.5691
Allergy**, n (%)	16 (43.2)	6 (16.2)	**0.0209**	17 (48.6)	5 (33.3)	0.3673	12 (33.3)	22 (44)	0.3751
Neurologic disease, n (%)	0	4 (10.8)	0.1148	0	0	NA	2 (5.6)	0	0.1724
Smoker, n (%)	8 (21.6)	9 (24.3)	1	6 (17.1)	4 (26.7)	0.4616	6 (16.7)	10 (20)	0.7836

***Table 1*: Demographic and baseline characteristics of COVID-19 patients and vaccinated controls.**

Abbreviation: BMI, Body mass index; NA, Not applicable.

* Metabolic conditions include insulin resistance, prediabetes, type 1/2 diabetes, non-alcoholic steatohepatitis and obstructive sleep apnea;

** Allergy considered self-reported allergic rhinitis (by seasonal, perennial/year-round, or episodic allergens) and food allergy. [Fisher's exact test; Mann Whitney test].

CoV-2, allows for rapid quantification, it enters cells through pathways of SARS-CoV-2, and does not require high biosafety containment. Briefly, Vero E6 cells (ATCC # CRL-1586, RRID:CVCL_0574) grown in 1X MEM (Gibco #11095-080) supplemented with 10% FBS (Gibco, #16000-044) were transfected with plasmid pCEP4-myc-ACE2 (Addgene catalog # 141185) and stable clones were selected by hygromycin (Invitrogen #10687010) (400 μg/mL). To assay nAb titres, serial dilutions of serum samples were incubated with rVSV-SARS2-S for 1 h at 37 °C. The serum-virus inoculum was added to Vero E6 hACE2 cells seeded the day before in optical bottom 96-well plates (Thermo Scientific #165305) at 80% confluence and adsorbed for 2 h at 37 °C. Next, the mixture was replaced by culture media and infection allowed to proceed for 20 h at 37 °C, 5% CO_2 and 80% humidity. The cells were then fixed with 4% formaldehyde (Pierce #28906) and stained in with 4′,6-diamidino-2-phenylindole (DAPI) 300 nM (Invitrogen #D1306). Viral infectivity was quantified by automated enumeration of GFP-positive cells (normalizing against cells stained with DAPI) using a Cytation5 automated fluorescence microscope (BioTek) and segmentation algorithms applied from the ImageJ program. Alternatively, total GFP fluorescence per well was acquired using the Cytation5 fluorescence lector (wavelength for DAPI 360 nm for absorption, 460 nm for emission and for GFP, 485 nm for absorption, 526 nm for emission) and normalized against DAPI fluorescence. The half-maximum inhibitory concentration (IC50) of the sera, were calculated from data obtained with two technical replicates using non-linear regression analysis and the curve fitting was done using second-order polynomial (quadratic); and linear regression models (using log10 IC50 transformed data) were done with GraphPad Prism 5 software.

Statistical analysis

We used a convenience sampling approach and included n = 37 outpatients and n = 37 hospitalized SARS-CoV-2 individuals from a total pool of 168 recruited individuals representative of the population of the Metropolitan region of the country. Of the 74 infected individuals, we included all those that were vaccinated through the national COVID-19 immunization campaign during the longitudinal follow up period, and hence, there were no a priori criteria for selecting these individuals. A convenience sampling of uninfected individuals (n = 50) that were voluntarily vaccinated through the national COVID-19 immunization campaign, were also invited to participate in the study. The samples were assigned an anonymous code and all serological analyses were performed by scientists that were blinded in regards to the subject's clinical condition and time of sample collection. Our study did not have any a priori exclusion criteria and hence all individuals with a laboratory confirmed SARS-CoV-2 infection or that had been vaccinated during the study period were invited to participate in the study. Categorical variables were expressed as numbers or percentages. Association between categorical variables was examined with Chi-squared or Fisher's exact test. Continuous variables were expressed in mean, geometric mean and range and compared with unpaired two-tailed Mann-Whitney test. Correlation was evaluated calculating the Pearson correlation coefficient. GraphPad Prism 8 was used for statistical analysis: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. We evaluated for potential cofounding effects on vaccinated individuals by first performing univariate analysis (Fisher's exact test) on all the demographics and clinical features. We then performed logistic regression with all the demographic variable and comorbidities. Variables that were found to be significant were used to perform a multivariate analysis, where the variables age and sex were included as common potential cofounder variables affecting immune responses. Relevant multivariate analyses were plotted as crude and adjusted odds ratio (OR) for vaccine responder capacity.

Ethics

Patient clinical and epidemiological data, along with their clinical specimens were collected after informed written consent was obtained under protocols 16-066 and 200829003, which were reviewed and approved by the Scientific Ethics Committee for Health Sciences (CECSaludUC, by its Spanish acronym) at Pontificia Universidad Católica de Chile (PUC).

Role of funders

The funders of this work had no role in the study design, management, data collection, data analysis, interpretation of the data nor the preparation, review, or approval of this manuscript and decision to submit the manuscript for publication.

Results

Longitudinal antibody titres induced by natural SARS-CoV-2 infections

To understand the long-term dynamics of antibody induction and decay after natural SARS-Cov-2 infection, we prospectively enrolled 74 individuals (overall mean age: 44 years [range 14–83, >60 23%]), of whom 37 were outpatient (mild disease mean age: 37 years [range 14–66]) and 37 were hospitalized (moderate [n = 23] and severe disease [n = 14], mean age: 51 years [range 16–83]) with a confirmed SARS-CoV-2 quantitative RT-PCR test. These individuals were followed longitudinally for up to 13·6 months from the onset of symptoms (demographic and baseline characteristics of the

patients are summarized in Table 1; convalescent samples were collected between 2 and 414 days after the onset of symptoms).

To analyse humoral responses longitudinally, to determine nAb titres we used a microneutralization assay based on a recombinant vesicular stomatitis virus carrying a SARS-CoV-2 spike protein,[22] that showed strong correlation (Pearson's r=0.80, R^2 = 0.65, $p < 0.001$) with authentic SARS-CoV-2 microneutralization (Supplementary Figure 4a,b), and evaluated the induction of anti-N IgG antibodies by ELISA. Regardless of disease severity and age, infected individuals developed robust nAb and anti-N IgG responses during the first month. The nAb responses declined over time but were sustained for up to 13.6 months (Figure 1a,b), whereas the anti-N IgG titres were detectable at least for 9 months (Figure 1c,d). We performed kinetic analyses with samples from 65 individuals that were sampled weekly during the first month from symptom onset (Figure 1a,c; Supplementary Figure 4c, d). In agreement with previous reports,[23,24] hospitalized individuals had significantly higher neutralization titres as compared to outpatients; with peak average nAb responses at day 25 and at day 23 post-symptom onset, respectively (Figure 1a and Supplementary Figure 4c,d). Similarly, the anti-N IgG titres peaked on days 23 for the hospitalized and day 22 for the outpatient individuals (Figure 1c). We included long-term longitudinal samples for 67 participants, which included samples from 58 individuals that were also analysed during the first month (Figure 1a) and performed nAb and anti-N IgG titre time decay analysis starting from the respective peak average responses (Figure 1a,b). Fitting our nAb data to a continuous decay model, estimated a half time of 147 days (95% CI = 68.7–322.5 days) for outpatients and 112 days (95% CI = 76.7–208.1 days) for hospitalized individuals (Figure 1b). For the anti-N IgG levels, the decay model for hospitalized individuals was 118 days (95% CI = 81–219.9 days) and for outpatients 600 days (95% CI = 203.8–635.6 days, Figure 1d). We then also compared longitudinally the antibody titres between hospitalized and outpatient individuals. Hospitalized individuals had significantly higher titres of nAbs at weeks 2–4 and at 3–9 months. However, between week four and month nine, the nAb GMT decrease of hospitalized individuals was 15 times compared to three times for outpatients (Figure 2a). A similar trend was observed when we assessed the anti-N IgG titres, which were significantly induced and remained higher in hospitalized individuals as compared to outpatients for the first 3 months since onset of symptoms (Figure 2b). However, these antibodies in the hospitalized group showed no significant differences to those observed for outpatients after 6 months. Noteworthy, in some individuals we detected basal levels of anti-N IgGs, above the limit of detection (LOD) but below the limit of sensitivity (LOS) for the assay, which suggest a potential previous exposure to seasonal coronaviruses that have shown to induce cross-reactive anti-N antibodies (e.g. HKU1 or OC43 strains).[6] None of the individuals in the study had evidence of re-infections. Taken together, while the nAb and anti-N IgG titres remained higher in the hospitalized patients, these individuals had a more pronounced decrease over time. Nonetheless, despite this decrease in titres in both study groups, all individuals showed long-lasting responses of circulating nAbs 9–13.6 months after natural infection.

Antibody responses in previously-infected individuals after vaccination

Thirty-six of the previously infected individuals (mean age: 44 years [range 17–83]) included in our longitudinally cohort were immunized during the study period (Figure 3), while 38 individuals were not immunised (Supplementary Figure 5). Thus, we analysed the nAb and anti-N IgG response in these previously infected individuals after immunization with the two main vaccines used in Chile; the CoronaVac (Sinovac) or the BNT162b2 (BioNTech/Pfizer) vaccines. The previously infected individuals were vaccinated between 4.2 and 13.3 months (average 9.7 months, Supplementary Figure 2 and Supplementary Table 1) after the onset of symptoms for both, the outpatient (20 participants, Figure 3a,b) and hospitalized groups (16 participants, Figure 3c,d). Except for three cases, all the previously infected participants showed an increase in the nAb titres after receiving one or two doses of the vaccines, suggesting a significant induction of B cell memory response months after onset of symptoms. Strikingly, the only three individuals (age range 29–63 years) that lacked an induction of nAbs responses were obese (3/10 obese participants), including an outpatient (Figure 3a, light green patient) or two hospitalized participants (Figure 3c, grey and cyan patients). For these individuals we only had a previous sample 5.6 to 10.7 months prior to vaccination (Figure 3a–c), and hence no clear conclusions can be drawn about the trajectory of their nAb titres. Noteworthy, one of these participants had a marked decrease in nAb titre after two doses of the vaccine (IC50 563.9 to 31.0; Figure 3c, cyan patient). Univariate analysis of obesity as a cofounding factor for responding to vaccination in the previously infected group showed statistical significance (Table 1). Unexpectedly, regardless of the time of vaccination or severity anti-N IgG were only modestly boosted and in only in some previously infected patients upon immunization with the CoronaVac vaccine (Figure 3b–d).

To establish statistical comparisons of the antibodies induced by immunization in previously infected individuals with these two widely used vaccines, we grouped the nAbs and anti-N IgG titres before and after being

vaccinated with CoronaVac or BNT162b2, and related them to antibody titres which these patients had reached during convalescence (Figure 4a,b). Given that there is yet no clear definition of the time frame in which protective nAb titres during convalescence should be considered, we took the peak maximal titres from our own data and its longitudinal decay, as well as the normalized data reported by Khoury et al.[12] No statistical differences were observed when we considered days ranges from 14 to 21, 14 to 28 and 10 to 37 since the initiation of symptoms (Supplementary Figure 3). Hence, we included the more dogmatic seroconversion data for the first 14-28 days post-infection for outpatients and hospitalized individuals (outpatients GMT = 681.2 [95% CI = 430.7–1077 GMT]; hospitalized GMT = 3232 [95% CI = 1984–5,266 GMT]; Figure 4a–c). There were only 7 samples available from previously infected individuals vaccinated with one dose of CoronaVac, therefore analysis of additional samples would be needed to further evaluate the boosting capacity of a single dose. After the second dose of the CoronaVac vaccine in previously infected individuals, the average nAb increase since the pre-vaccine time point was 12 times among outpatients (pre-vaccine GMT = 174 [95% CI = 81.2–372 GMT], second dose GMT = 2057.3 [95% CI = 987.7–4,285 GMT]) and five times among hospitalized (pre-vaccine GMT=700.8 [95% CI=171.9–2,856.8 GMT], second dose GMT = 2113.6 [95% CI = 412.9–10,018.7 GMT]; Figure 4a). When compared to the 14–28 day convalescent titre, the pre-vaccination titres (Vacc Dose 0) of outpatients and hospitalized individuals were significantly lower. However, only the previously infected outpatients group immunized with two doses of CoronaVac generated a significant increase in titre, which re-established them to levels comparable to the convalescent titres (Figure 4a). In general, hospitalized individuals had sustained higher antibody levels at the time of vaccination, however; while immunization with CoronaVac generated a measurable nAb titre increase in most of these individuals, the overall level of induction was not significant (Figure 4a).

When we assessed the induction of anti-N IgG of these previously infected individuals after immunization with CoronaVac, surprisingly there was no increase in titre in the outpatients as compared to their convalescent levels, and only a modest increase in titres was observed in the hospitalized group (Figure 4b), suggesting that this inactivated virus vaccine is a poor inducer of anti-N antibodies. There were only 6 cases of previously infected individuals that were immunized with BNT162b2 (3 outpatient and 3 hospitalized) and therefore we had insufficient statistical power to perform any further analyses. Nonetheless, as previously reported, the general pattern in these individuals showed an induction in their nAbs (Figure 4a)[25] and as expected no increases in anti-N IgGs were observed (Figure 4b).

Induction of antibody responses in naïve individuals through vaccination

To compare the antibody titres of previously infected individuals at convalescence and after vaccination to those of healthy naÿve (SARS-CoV-2 seronegative) individuals immunized with one and two doses of either vaccine representing similar demographic characteristic (CoronaVac, 35 participants, mean age: 36 years [range 21–80] or BNT162b2, 15 participants, mean age: 34 years [range 15–53]; Figure 4c,d, Table 1), we determined the overall GMT antibody titre of both groups; outpatients (OP) and hospitalized (HP) individuals. For a broader point of comparison and to establish significant differences among all groups, in our analyses we also included the combined 14-28 day convalescent antibody titres from all previously infected individuals (Total, Figure 4c,d) representing the broad diversity of nAbs after natural infection (14–28 days GMT = 1596.9).

The induction of nAbs in naÿve individuals vaccinated with CoronaVac (one dose GMT = 21.9; two doses GMT = 311.9) were lower to those of the combined titres of convalescent patients (Figure 4c). These lower levels were highly significant when we compared to those of hospitalized individuals (GTM = 3232.2) and to a lesser extent when compared to the outpatient group (GTM=681.2). Overall, this indicated that the nAb levels induced by CoronaVac were significantly lower to those generated after natural infection (Figure 4c), likely due to the high levels of viral replication in infected individuals.[24] In addition, three out of 35 individuals immunized with CoronaVac did not seroconvert. Naÿve individuals vaccinated with BNT162b had similar nAb titres after one dose (GMT = 465.7), but much higher titres after two doses (GMT = 8387.5) as compared to convalescent patients. This also indicated that one dose of the BNT162b vaccines induces similar nAb levels than immunization with two doses of CoronaVac. On the other hand, individuals with two doses of the BNT162b vaccine reached levels that were significantly higher to those of the convalescent outpatients and hospitalized combined, being most similar to those titres observed in the hospitalized group at convalescence or after these individuals were immunized with two doses of CoronaVac (Figure 4c). When we evaluated the induction of anti-N IgG through immunization with CoronaVac in naÿve individuals, there were only a few individuals that had a detectable increase in titres after the second dose (Figure 4d). The overall anti-N IgG titres in vaccinees were significantly lower as compared to the combined or hospitalized convalescent titre but similar to that induced in convalescent outpatients after natural infection. As expected, no variation in anti-N titres was observed in individuals immunized with the BNT162b vaccine.

Since three naÿve individuals did not respond to immunization, we assessed for potential cofounding

factors affecting vaccine response. There were no demographic or clinical variables associated with either seroconvertion or lack of antibody induction in the naÿve immunized group. We further analyzed all the vaccinee data, by including the previously infected and naÿve-vaccinated participants together, two variables associated with a lack of vaccine response, age and obesity (Supplemental Table 2). Remarkably, logistic regression and multivariate analyses confirmed obesity as an underlying comorbidity affecting vaccine response (Supplemental Figure 6).

Neutralizing levels induced by CoronaVac and BNT162b2 vaccines and estimates of predictive efficacy

To assess the association of the nAbs titres from our study to the reported protection by the CoronaVac and BNT162b2 vaccines, we used the logistic models of Khoury et al.[12] and Earle et al.[13]. In these models, the nAbs titres of the different studies were normalized to the mean convalescent titres of the same study, and compared against the corresponding protective efficacy reported from the phase 3 clinical trials. Hence, to analyse our data with these models, we calculated the mean neutralization level induced by the vaccines as a fold comparison to the combined convalescent titres of individuals at 14-28 days post-symptom onset (GMT = 1597). The mean titre induced by two doses of CoronaVac was 0·2 times that of convalescent individuals, whereas two doses of the BNT162b vaccine resulted in 5.25 times, representing a highly significant difference in the neutralization levels induced by both vaccines. By extrapolating these data to the mathematical models, the estimated predicted efficacy for CoronaVac was ~50% and for BNT162b was ~97%, suggesting that our independent data confirms the difference in predictive protection reported previously for both vaccines.[26,27]

Discussion

We found long-lasting nAb titres that persist for at least 13.6 months after the onset of symptoms in both, outpatient and hospitalized individuals. This is in agreement with the detection of SARS-CoV-2 spike-specific long-lived bone marrow plasma cells 7-8 months post-symptom onset.[28] Our cohort study provides empirical data showing that long-lasting nAb responses induced through natural infection can be significantly boosted after immunization with CoronaVac or BNT162b2 vaccines, when administered up to 13.3 months since the onset of COVID-19 symptoms, suggesting that infection induces a robust B-cell memory response. Such responses have been well characterized in infected individuals[28,29] and are also critical for the durability of protection in vaccinated individuals.[30,31] Importantly, the decay of nAbs titres after infection seen over time in our study and reported by other groups,[3,12,28] suggests that booster immunization strategies of previously infected individuals should be considered and might be required to control the pandemic and prevent re-infection with new variants of concern (VOC) in subsequent years.

Our longitudinal data indicates that infected individuals generated robust nAb and anti-N IgG titres. Interestingly, hospitalized individuals had significantly higher titres when evaluated longitudinally throughout the study period, which is likely due to sustained higher viral loads observed in these individuals.[24] There was a more pronounced decay of both antibody titres in the hospitalized population as compared to the outpatient group. Of note, individuals in the hospitalized group were ~14 years older, which might explain the faster decay in this group (Figure 1 and Table 1). While nAbs were significantly boosted with two doses of CoronaVac in the previously infected group, surprisingly there was no or only moderate induction of anti-N IgG in any of the previously infected individuals (Figs. 3 and 4). Similarly, there was poor induction of anti-N antibodies after vaccination of naÿve individuals with CoronaVac, overall confirming that this vaccine is a poor inducer of antibody responses against this protein.[32–34] This is important to note, given that the protection afforded by CoronaVac is most likely due to the induction of nAbs responses, and additional immune mechanisms such as Fc-effector functions and T-cell immunity, which contribute to improve disease outcome.[15,16]

The correlates of protection against SARS-CoV-2 are currently unknown. However, current evidence of re-infections with the same virus variant remains limited.[7,35–37] However this is a situation that continues to evolve given the emergence of VOCs such as Gamma, and Delta, which have shown significant reduction in cross-reactive neutralizing titre, and have generated increased rates of re-infection in some regions of the world,[38–40] a scenario that remains to be fully evaluated with the new VOC, Omicron. To further strengthen models for protective correlates, additional comparative analyses of nAb titres of vaccinated individuals and better-defined standards for convalescent sera that incorporate titre variations due to disease severity and decay over time are needed. The current study provides a unique dataset and a direct comparison of longitudinal convalescent nAb titres to those of individuals immunized with two different vaccine formulations approved by the WHO and are currently being widely used. These comparative data is of crucial value to establish the relationship between neutralization level and efficacy against symptomatic SARS-CoV-2 infection, as recently proposed.[12,13]

The calculated mean GMT induced by vaccination of naÿve individuals with CoronaVac and BNT162b2 were 0.2 and 5.5 times, respectively, that of convalescent titres

at 14–28 days post-symptom onset. This suggests that the antibody response in previously uninfected individuals vaccinated with CoronaVac was significantly lower compared to individuals who have recovered from SARS-CoV-2 infection.[2,3,41] As shown, more severe individuals had higher antibody titres, which other studies have been associated with increased replication and disease burden.[24] Although lower titres are seen in Coronavac vaccinated individuals, as compared to natural infected individuals, this data indicates that immunization by this vaccine affords protection from COVID-19, as shown in recent vaccine effectiveness studies in Chile.[42] The BNT162b2 vaccine induced titres that were higher and more similar to the titre of hospitalized convalescent patients. Based on these titres, the predicted efficacy by the mathematical model of Khoury et al. and Earle et al. suggested a ~50% protection from symptomatic disease for CoronaVac and 97% for BNT162b2. While this predicted efficacy coincide well with the reported phase 3 trial of 95% for BNT162b2,[43] for the CoronaVac vaccine the 50% prediction is lower compared to clinical data showing protection of 50–84% depending on the geographic location.[11] Interestingly in our study, we determined nAb in sera collected from vaccinated individuals at 20–30 days post first dose and at 13–19 days after the second dose since immunization. The large real-life effectiveness data reported from Chile for BNT162b2 was 92.6% and for CoronaVac was 62.8–64.6%,[42] which considered a similar timing post vaccination to evaluate effectiveness (e.g. those individuals who were partially immunized [≥14 days after receipt of the first vaccine dose and before receipt of the second dose], and those who were fully immunized [≥14 days after receipt of the second dose] allowing us to compare both parallel results. In contrast, in the CoronaVac clinical trial from Turkey, which represented a smaller sample size and included a large number of elderly individuals, among other differences, the reported protection was as high as 84%.[44] While the prediction models correlated fairly well with the observed efficacy for most vaccines, Khoury et al. reported a less optimal correlation for the CoronaVac vaccine as these data points were towards the lower end of the logistic model. Hence, additional data such as the data provided from this study along side with real life efficacy data, may strengthen such models. Our study indeed suggests that lower nAb titres might still afford protection from disease. Moreover, approximately 10% of the individuals vaccinated with CoronaVac did not seroconvert, which has also been reported by others.[45] This is in line with the notion that even lower nAb titres can be sufficient to protect from severe disease.[12] Hence, additional cellular immune responses, such as T-cell immunity and Fc-effector mechanisms might also contribute significantly to protection. Thus, additional assessment of the correlates of protection induced by this and other vaccines warrants further investigations. Of note, our analyses revealed that obesity was a risk factor affecting seroconversion after immunization (Table 1 and Supplemental Figure 6 and Supplemental Table 2). Obesity has been reported to be a comorbidity associated with an increased risk of developing severe COVID-19,[46,47] and increased body mass index (BMI) has been associated with decreased IgG levels.[48] Hence, further studies to monitor the induction and decay of nAbs after vaccination in this population are needed.

Our study has some limitations. Firstly, it is a small longitudinal cohort representing a limited number of individuals tested out of the population diagnosed with COVID-19 in Chile during the study period. Moreover, it is currently uncertain how these results compare to the overall antibody levels induced by the CoronaVac and BTN162b2 vaccines in the general population, and hence, a larger study would be needed to draw further conclusions. In addition, we used a convenience sampling approach to rapidly recruit naÿve vaccinated individuals. While the overall demographics of this group was highly similar to the previously infected immunized group, the average age of the naÿve group was 9 years younger. Given that age is a known factor affecting vaccination response (Supplemental Figure 6), further assessment of the effect of age, obesity and other comorbidities in vaccines response in the general population are warranted. In addition, at the time of this study Chile had vaccinated >75% of its population, mainly with CoronaVac (https://deis.minsal.cl/), and saw a drastic reduction of COVID-19 cases (epidemiological weeks 24–31), even while the predominant circulating variants were Gamma (P.1 VOC; at 75% frequency) and Lambda (C.37 variant of interest; at 20% frequency) (https://vigilancia.ispch.gob.cl/app/varcovid). Noteworthy, in South America and Chile, there seemed to be distinct dynamics (apparently delayed) of the introduction of the Delta VOC as compared to other countries in the Northern Hemisphere. Thus, our data suggest that the immunity (humoral and B cell memory) induced by immunization with CoronaVac in the general population was capable of reducing the circulation of the SARS-CoV-2 strains including two recently emerged variants. However, this data also suggest that a larger proportion of the population would need to be immunized with CoronaVac to have an impact in the circulation of the virus and afford community level immunity, as compared to other vaccines. In fact, Chile has now (epidemiological week 9, 2022) reached >93% of its population vaccinated, and saw a very small peak of the Delta VOC in mid-November (epidemiological week 47) and a large peak of the Omicron VOC, but with reduced hospitalizations and severe cases. Nonetheless, Chile has also used other vaccines (Ad5-nCoV; Cansino, ChAdOx1 nCoV-19; AstraZeneca) and started to offer booster doses of BNT162b and ChAdOx1 nCoV-19 in mid-August (epidemiological week 33, 2021) to all

individuals vaccinated 6 months earlier (https://deis.minsal.cl/). Hence, the direct independent effect of CoronaVac on herd immunity cannot be estimated.

Booster vaccination schemes seem fundamental, especially when the nAb decay over time is taken into consideration, as shown in our longitudinal study of infected individuals (half times ~112 to ~147 days) or longitudinal vaccine cohort studies.[49] Such a decay is of particular concern when considering that the CoronaVac vaccine induces low initial nAb titers. This suggests that vaccination with CoronaVac will require booster doses within shorter time frames as compared to other vaccines, and therefore this data contributes to further defining the proper strategies and timing to implement boost immunizations for the general population.[10]

The WHO Strategic Advisory Group of Experts on Immunization (SAGE) in June 1st, 2021 authorized the CoronaVac for emergency use (https://www.who.int/news/item/01-06-2021-who-validates-sinovac-covid-19-vaccine-for-emergency-use-and-issues-interim-policy-recommendations). In this context, our data are highly relevant for the COVAX initiative and the developing world (e.g. 50 countries that have already authorized CoronaVac, including being extensively used in Chile, Turkey, Brazil, China and Indonesia). Further studies to determine and monitor the long-term duration of nAbs against SARS-CoV-2 induced by different vaccine formulations and against emergent variants are warranted.

Contributors

NAM and TGS collected and analyzed data, made figures and tables, interpreted data, and wrote the paper. CPR, EFS, JL, MJA, LIA, EP, and SS processed samples, performed experiments, analyzed data, and revised the paper. GV, ES, AM, CG, AR, recruited patients, collected clinical metadata, and revised the paper. RJ, KC, DH, MED, generated rVSV viral stocks, analyzed data and revised the paper. FK analyzed serological data, advised on data interpretation, and provided funding for the study and revised the paper. NT designed the study, collected, analyzed, and interpreted data, provided funding for the study, and wrote the paper. RAM conceived the longitudinal cohort design, recruited patients; collected, analyzed, and interpreted data, provided funding for the study, and wrote the paper. Data was verified by NAM, TGS. NT and RAM and made the decision to submit this manuscript. All authors read and approved the final version of the manuscript.

Data sharing

The individual-level data used in this study are sensitive and cannot be publicly shared. The data sets generated and that support the findings of this study are available from the corresponding authors on reasonable request.

Declaration of interests

The authors reported no potential conflict of interest. The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines which list Florian Krammer as co-inventor. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. Florian Krammer has consulted for Merck and Pfizer (before 2020), and is currently consulting for Pfizer, Seqirus, Avimex and Third Rock Ventures. The Krammer laboratory is also collaborating with Pfizer on animal models for SARS-CoV-2. Kartik Chandran is a member of the scientific advisory boards of Integrum Scientific, LLC Biovaxys Technology Corp, and Celdera Medical, LLC; has received royalties from Q2 Solutions and has consulted for Axon Advisors, LLC. Denise Haslwanter, Maria Eugenia Dieterle, Rohit K Jangra and Kartik Chandran, are listed as inventors on a patent application covering the VSV-based SARS2 neutralization assay assigned to Albert Einstein College of Medicine. Rafael Medina has received funding from NIH-Centers of Excellence for Influenza Research and Response (CEIRR) Contract HHSN 75N9301R00028, NIH-Centers of Excellence for Influenza Research and Surveillance (CEIRS) - HHSN272201400008C, the Hope COVID-19 initiative, BHP – UC, FONDECYT 1212023 – ANID Chile and the NIH-NIAID 1U19AI135972: Fluomics: The Next Generation.

Acknowledgements

We would like to thanks Luis A. Diaz for excellent clinical assistance during the study period, Zandra Segovia for assistance in patient recruitment and follow-ups and to all individuals that selflessly participated in this study. We also express our deepest gratitude to all the individuals that participated in this study. Work in the Tischler Laboratory was partially funded by FONDECYT 1181799 and Centro Ciencia & Vida, FB210008, Financiamiento Basal para Centros Científicos y Tecnológicos de Excelencia grants from the Agencia Nacional de Investigación y Desarrollo (ANID) of Chile. Work in the Medina Laboratory was based on protocols and the study set-up used for influenza virus established in part with the support of the PIA ACT 1408, FONDECYT 1161971, 1212023, and CONICYT REDES180170 grants from ANID of Chile, the FLUOMICS Consortium (NIH-NIAD grant U19AI135972) and the Center for Research on Influenza Pathogenesis (CRIP), an NIAID Center of Excellence for Influenza Research and Surveillance (CEIRS, contract # HHSN272201400008C) to RAM and FK. Additionally, work in the Krammer laboratory was partially funded by the NIAID Collaborative Influenza Vaccine Innovation Centers (CIVIC) contract 75N93019C00051, by the generous support of the JPB Foundation and the Open Philanthropy Project (research grant 2020-215611 (5384); and by anonymous

donors. Work in the Chandran laboratory was partially supported by NIH grant R01AI132633. CPR and JL conducted this work as part of their Postdoctoral grants FONDECYT 3190706 and 3190648, respectively. MJA and ES conducted this work as part of their Ph.D. Thesis, under Programa de Doctorado en Ciencias Biológicas mención Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. MJA was funded by the ANID Becas/Doctorado Nacional 21212258 scholarship and ES was funded by a scholarship from Vicerrectoría de Investigación de la Escuela de Graduados, Pontificia Universidad Católica de Chile. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.103972.

References

1 Post N, Eddy D, Huntley C, et al. Antibody response to SARS-CoV-2 infection in humans: a systematic review. *PLoS ONE*. 2020;15:(12):e0244126. PubMed PMID: 33382764. PMCID: PMC7775097 www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; JM is chief scientific officer, shareholder and scientific founder of Leucid Bio, a spinout company focused on development of cellular therapeutic agents; no other relationships or activities that could appear to have influenced the submitted work. This does not alter our adherence to PLoS ONE policies on sharing data and materials. Epub 2021/01/01.

2 Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Science*. 2020;370(6521):1227–1230. Dec 4PubMed PMID: 33115920. PMCID: PMC7810037. Epub 2020/10/30.

3 Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science*. 2021;371(6529):1–13. Feb 5PubMed PMID: 33408181. PMCID: PMC7919858. Epub 2021/01/08.

4 Gaebler C, Wang Z, Lorenzi JCC, et al. Evolution of antibody immunity to SARS-CoV-2. *Nature*. 2021;591(7851):639–644. MarPubMed PMID: 33461210. Epub 2021/01/19.

5 Anand SP, Prevost J, Nayrac M, et al. Longitudinal analysis of humoral immunity against SARS-CoV-2 Spike in convalescent individuals up to 8 months post-symptom onset. *Cell Rep Med*. 2021;2(6):1–10. PubMed PMID: 33969322. PMCID: PMC8097665. Epub 2021/05/11.

6 Aydillo T, Rombauts A, Stadlbauer D, et al. Immunological imprinting of the antibody response in COVID-19 patients. *Nat Commun*. 2021;12(1):3781. PubMed PMID: 34145263. PMCID: PMC8213790. Epub 2021/06/20.

7 Krammer F. Correlates of protection from SARS-CoV-2 infection. *Lancet*. 2021;397(10283):1421–1423. 04PubMed PMID: 33844964. PMCID: PMC8040540. Epub 2021/04/09. eng.

8 Hodgson SH, Mansatta K, Mallett G, Harris V, Emary KRW, Pollard AJ. What defines an efficacious COVID-19 vaccine? A review of the challenges assessing the clinical efficacy of vaccines against SARS-CoV-2. *Lancet Infect Dis*. 2021;21(2):e26–e35. FebPubMed PMID: 33125914. PMCID: PMC7837315. Epub 2020/10/31.

9 Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Xing Z. Immunological considerations for COVID-19 vaccine strategies. *Nat Rev Immunol*. 2020;20(10):615–632. OctPubMed PMID: 32887954. PMCID: PMC7472682. Epub 2020/09/06.

10 Croda J, Ranzani OT. Booster doses for inactivated COVID-19 vaccines: if, when, and for whom. *Lancet Infect Dis*. 2021:430–432. PubMed PMID: 34890538. PMCID: PMC8651253 from the Pan American Health Organization. Epub 2021/12/11.

11 WHO S. *SAGE Working Group on COVID-19 Vaccines - Evidence Assessment: Sinovac/CoronaVac COVID-19 Vaccine*. World Health Organization; 2021. https://cdn.who.int/media/docs/default-source/immunization/sage/2021/april/5_sage29apr2021_critical-evidence_sinovac.pdf.

12 Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med*. 2021:1205–1211. PubMed PMID: 34002089. Epub 2021/05/19.

13 Earle KA, Ambrosino DM, Fiore-Gartland A, et al. Evidence for antibody as a protective correlate for COVID-19 vaccines. *Vaccine*. 2021;39(32):4423–4428. PubMed PMID: 34210573. PMCID: PMC8142841. Epub 2021/07/03.

14 WHO RD. *COVID-19 Vaccines WHO Meeting on Correlates of Protection - R&D Blueprint*. World Health Organization; 2021. https://cdn.who.int/media/docs/default-source/blue-print/final-agenda-immunobridging.pdf?sfvrsn=8e074908_17.

15 Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell*. 2021;184(4):861–880. PubMed PMID: 33497610. PMCID: PMC7803150 Epitogenesis, Gilead, and Avalia. S.C. is a consultant for Avalia. LJI has filed for patent protection for various aspects of T cell epitope and vaccine design work. Epub 2021/01/27.

16 Kaplonek P, Wang C, Bartsch Y, et al. Early cross-coronavirus reactive signatures of humoral immunity against COVID-19. *Sci Immunol*. 2021;6(64):eabj2901. PubMed PMID: 34652962. Epub 2021/10/16.

17 Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 seroconversion in humans: a detailed protocol for a serological assay, antigen production, and test setup. *Curr Protoc Microbiol*. 2020;57(1):e100. PubMed PMID: 32302069. PMCID: PMC7235504. eng.

18 Amanat F, Stadlbauer D, Strohmeier S, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med*. 2020;26(7):1033–1036. PubMed PMID: 32398876. Epub 2020/05/12. eng.

19 ter Meulen J, van den Brink EN, Poon LL, et al. Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of escape mutants. *PLoS Med*. 2006;3(7):e237. PubMed PMID: 16796401. PMCID: PMC1483912. eng.

20 Tian X, Li C, Huang A, et al. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. *Emerg Microbes Infect*. 2020;9(1):382–385. PubMed PMID: 32065055. PMCID: PMC7048180. Epub 2020/02/17. eng.

21 Amanat F, White KM, Miorin L, et al. An *in vitro* microneutralization assay for SARS-CoV-2 serology and drug screening. *Curr Protoc Microbiol*. 2020;58(1):e108. PubMed PMID: 32585083. PMCID: PMC7361222. Epub 2020/06/26.

22 Dieterle ME, Haslwanter D, Bortz RH, et al. A replication-competent vesicular stomatitis virus for studies of SARS-CoV-2 spike-mediated cell entry and its inhibition. *Cell Host Microbe*. 2020;28(3):486–496. 09e6. PubMed PMID: 32738193. PMCID: PMC7332447. Epub 2020/07/03. eng.

23 Legros V, Denolly S, Vogrig M, et al. A longitudinal study of SARS-CoV-2-infected patients reveals a high correlation between neutralizing antibodies and COVID-19 severity. *Cell Mol Immunol*. 2021;18(2):318–327. FebPubMed PMID: 33408342. PMCID: PMC7786875. Epub 2021/01/08.

24 Carvalho T, Krammer F, Iwasaki A. The first 12 months of COVID-19: a timeline of immunological insights. *Nat Rev Immunol*. 2021;21(4):245–256. AprPubMed PMID: 33723416. PMCID: PMC7958099. Epub 2021/03/17.

25 Krammer F, Srivastava K, Alshammary H, et al. Antibody responses in seropositive persons after a single dose of SARS-CoV-2 mRNA vaccine. *N Engl J Med*. 2021;384(14):1372–1374. PubMed PMID: 33691060. PMCID: PMC8008743. Epub 2021/03/10. eng.

26 Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis*. 2021;21(2):181–192. PubMed PMID: 33217362. PMCID: PMC7832443. Epub 2020/11/21.

27 Walsh EE, Frenck RW, Falsey AR, et al. Safety and Immunogenicity of two RNA-based COVID-19 vaccine candidates. *N Engl J Med*. 2020;383(25):2439–2450. PubMed PMID: 33053279. PMCID: PMC7583697. Epub 2020/10/15.

28 Turner JS, Kim W, Kalaidina E, et al. SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans. *Nature*. 2021:421–425. PubMed PMID: 34030176. Epub 2021/05/25.

Articles

29 Sakharkar M, Rappazzo CG, Wieland-Alter WF, et al. Prolonged evolution of the human B cell response to SARS-CoV-2 infection. *Sci Immunol*. 2021;6(56):1–14. PubMed PMID: 33622975. PMCID: PMC8128290. Epub 2021/02/25.

30 Goel RR, Apostolidis SA, Painter MM, et al. Distinct antibody and memory B cell responses in SARS-CoV-2 naive and recovered individuals following mRNA vaccination. *Sci Immunol*. 2021;6(58):1–13. PubMed PMID: 33858945. PMCID: PMC8158969. Epub 2021/04/17.

31 Sokal A, Barba-Spaeth G, Fernandez I, et al. mRNA vaccination of naive and COVID-19-recovered individuals elicits potent memory B cells that recognize SARS-CoV-2 variants. *Immunity*. 2021;54 (12):2893–2907. PubMed PMID: 34614412. PMCID: PMC8452492. Epub 2021/10/07.

32 Benjamanukul S, Traiyan S, Yorsaeng R, et al. Safety and immunogenicity of inactivated COVID-19 vaccine in health care workers. *J Med Virol*. 2021:1442–1449. PubMed PMID: 34783049. PMCID: PMC8661929. Epub 2021/11/17.

33 Azak E, Karadenizli A, Uzuner H, Karakaya N, Canturk NZ, Hulagu S. Comparison of an inactivated COVID19 vaccine-induced antibody response with concurrent natural Covid19 infection. *Int J Infect Dis*. 2021;113:58–64. PubMed PMID: 34597764. PMCID: PMC8479817. Epub 2021/10/02.

34 Keskin AU, Bolukcu S, Ciragil P, Topkaya AE. SARS-CoV-2 specific antibody responses after third CoronaVac or BNT162b2 vaccine following two-dose CoronaVac vaccine regimen. *J Med Virol*. 2022;94(1):39–41. PubMed PMID: 34536028. Epub 2021/09/19.

35 Jeffery-Smith A, Rowland TAJ, Patel M, et al. Reinfection with new variants of SARS-CoV-2 after natural infection: a prospective observational cohort in 13 care homes in England. *Lancet Healthy Longev*. 2021;2(12):e811–e819. PubMed PMID: 34873592. PMCID: PMC8635459 England, which is a public body and an executive agency of the Department of Health and Social Care. Epub 2021/12/08.

36 Vitale J, Mumoli N, Clerici P, et al. Assessment of SARS-CoV-2 reinfection 1 year after primary infection in a population in Lombardy, Italy. *JAMA Intern Med*. 2021;181(10):1407–1408. PubMed PMID: 34048531. PMCID: PMC8164145. Epub 2021/05/29.

37 Qureshi AI, Baskett WI, Huang W, Lobanova I, Naqvi SH, Shyu CR. Re-infection with SARS-CoV-2 in patients undergoing serial laboratory testing. *Clin Infect Dis*. 2021:294–300. PubMed PMID: 33895814. PMCID: PMC8135382. Epub 2021/04/26.

38 Buss LF, Prete CA, Abrahim CMM, et al. Three-quarters attack rate of SARS-CoV-2 in the Brazilian Amazon during a largely unmitigated epidemic. *Science*. 2021;371(6526):288–292. PubMed PMID: 33293339. PMCID: PMC7857406. Epub 2020/12/10.

39 Sabino EC, Buss LF, Carvalho MPS, et al. Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. *Lancet*. 2021;397 (10273):452–455. PubMed PMID: 33515491. PMCID: PMC7906746. Epub 2021/01/31.

40 Chemaitelly H, Bertollini R, Abu-Raddad LJ. National study group for C-E. Efficacy of natural immunity against SARS-CoV-2 reinfection with the beta variant. *N Engl J Med*. 2021;385(27):2585–2586. PubMed PMID: 34910864. PMCID: PMC8693689. Epub 2021/12/16.

41 Gillot C, Favresse J, Maloteau V, Dogne JM, Douxfils J. Dynamics of neutralizing antibody responses following natural SARS-CoV-2 infection and correlation with commercial serologic tests. A reappraisal and indirect comparison with vaccinated subjects. *Viruses*. 2021;13(11):1–9. PubMed PMID: 34835135. PMCID: PMC8621742. Epub 2021/11/28.

42 Jara A, Undurraga EA, Gonzalez C, et al. Effectiveness of an Inactivated SARS-CoV-2 vaccine in Chile. *N Engl J Med*. 2021;385 (10):875–884. PubMed PMID: 34233097. PMCID: PMC8279092. Epub 2021/07/08.

43 Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N Engl J Med*. 2020;383 (27):2603–2615. PubMed PMID: 33301246. PMCID: PMC7745181. Epub 2020/12/11.

44 Tanriover MD, Doganay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *Lancet*. 2021;398(10296):213–222. PubMed PMID: 34246358. PMCID: PMC8266301. Epub 2021/07/12.

45 Bueno SM, Abarca K, Gonzalez PA, Galvez NMS, Soto JA, Duarte LF, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine in a subgroup of healthy adults in Chile. *Clin Infect Dis*. 2021:1–13. PubMed PMID: 34537835. Epub 2021/09/20.

46 Epsi NJ, Richard SA, Laing ED, et al. Clinical, immunological, and virological SARS-CoV-2 phenotypes in obese and nonobese military health system beneficiaries. *J Infect Dis*. 2021;224(9):1462–1472. PubMed PMID: 34331541. PMCID: PMC8385847. Epub 2021/08/01.

47 Huang Y, Lu Y, Huang YM, et al. Obesity in patients with COVID-19: a systematic review and meta-analysis. *Metabolism*. 2020;113: 154378. DecPubMed PMID: 33002478. PMCID: PMC7521361. Epub 2020/10/02.

48 Frasca D, Reidy L, Cray C, et al. Influence of obesity on serum levels of SARS-CoV-2-specific antibodies in COVID-19 patients. *PLoS ONE*. 2021;16(3):e0245424. PubMed PMID: 33760825. PMCID: PMC7990309. Epub 2021/03/25.

49 Tartof SY, Slezak JM, Fischer H, et al. Effectiveness of mRNA BNT162b2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA: a retrospective cohort study. *Lancet*. 2021;398(10309):1407–1416. PubMed PMID: 34619098. PMCID: PMC8489881. Epub 2021/10/08.

CoronaVac

O que a ciência comprova

1.3. Resposta imune induzida pela CoronaVac é superior à da infecção natural, mostra estudo da Turquia

Um estudo da Turquia publicado na revista Vaccine voltou a mostrar que, em pessoas que nunca tiveram Covid-19, a CoronaVac induz uma proteção maior do que a observada em quem só teve a infecção e não foi vacinado. Além disso, indivíduos com infecção prévia que tomam CoronaVac multiplicam a sua quantidade de anticorpos. O trabalho reforça a importância da imunização e se soma a outros estudos que já mostraram que a infecção natural não é suficiente para produzir resposta imune duradoura, já que a vacina induz uma proteção superior à provocada somente pela doença.

Conduzida na Faculdade de Medicina da Universidade de Marmara, em Istambul, a pesquisa incluiu 224 profissionais de saúde, divididos em quatro grupos: 75 voluntários sem histórico de Covid-19 que foram vacinados com CoronaVac; 53 que foram imunizados após terem tido a infecção; 60 com infecção prévia que não se vacinaram; e 36 que tiveram Covid-19 após a vacinação.

Todos os participantes do estudo apresentaram soroconversão (produção de anticorpos), sendo que os níveis de anticorpos IgG foram mais altos naqueles que tomaram a vacina após terem tido a doença (620 BAU/mL) e nos vacinados sem infecção prévia (136 BAU/mL), em comparação com aqueles que só tiveram a infecção e não foram imunizados.

O grupo com maior produção de anticorpos foi o de quem teve Covid-19 após a vacinação, com 6.146 BAU/mL, demonstrando que a vacina ativa a memória imunológica e potencializa a resposta do organismo contra a infecção. No entanto, esses dados não foram comparados estatisticamente porque o intervalo entre a segunda dose da vacina até a amostragem de sangue não correspondeu aos intervalos dos outros grupos.

"Os profissionais de saúde que receberam duas doses de CoronaVac antes e depois da infecção natural por SARS-CoV-2 tiveram uma resposta de anticorpos significativamente maior, comprovando a importância de um 'terceiro contato' com o vírus, ou seja, uma dose de reforço", apontam os autores.

A superioridade das vacinas em relação a infecções naturais é algo amplamente comprovado pela ciência. Um estudo recente feito na China mostrou que pessoas que tomaram CoronaVac e foram infectadas pela delta estão protegidas contra a ômicron, ao contrário de quem teve a doença e não se vacinou. Outra pesquisa conduzida na Turquia também demonstrou que indivíduos previamente infectados que foram vacinados com CoronaVac apresentam aumento no nível de anticorpos.

Somente a vacinação protege contra hospitalizações e mortes

Por ser uma vacina de vírus inativado, a CoronaVac "mimetiza" a infecção sem causar a doença. E como os estudos de efetividade feitos no mundo têm comprovado, a CoronaVac protege mais de 90% contra hospitalizações e mortes. Saiba mais sobre os benefícios da vacina do Butantan no Dossiê CoronaVac.

Publicado em: 18/03/2022

Vaccine 40 (2022) 2619–2625

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

The effect of immunization with inactivated SARS-CoV-2 vaccine (CoronaVac) and/or SARS-CoV-2 infection on antibody levels, plasmablasts, long-lived-plasma-cells, and IFN-γ release by natural killer cells

Huseyin Bilgin [a], Marisa Marku [a,1], Sultan Seval Yilmaz [b,1], Aysegul Karahasan Yagci [c], Uluhan Sili [a], Baris Can [c], Rabia Can Sarinoglu [c], Lutfiye Mulazimoglu Durmusoglu [a], Goncagul Haklar [b], Onder Sirikci [b,2,*], Emel Eksioglu Demiralp [d,2]

[a] Marmara University, School of Medicine, Department of Infectious Diseases and Clinical Microbiology, Istanbul, Turkey
[b] Marmara University, School of Medicine, Department of Biochemistry, Istanbul, Turkey
[c] Marmara University, School of Medicine, Department of Microbiology, Istanbul, Turkey
[d] Istanbul Memorial Şişli Hospital, Tissue Typing and Immunology Laboratory, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 20 November 2021
Received in revised form 25 February 2022
Accepted 1 March 2022
Available online 18 March 2022

Keywords:
COVID-19
SARS-CoV-2 infection
Whole virion inactivated vaccine
CoronaVac
IFN-gamma
Anti-spike antibody

ABSTRACT

Objectives: We evaluated the antibody response, natural killer cell response and B cell phenotypes in healthcare workers (HCW) who are vaccinated with two doses of CoronaVac with or without documented SARS-CoV-2 infection and unvaccinated HCWs with SARS-CoV-2 infection.
Methods: HCWs were divided into four groups: vaccine only (VO), vaccine after SARS-CoV-2 infection (VAI), SARS-CoV-2 infection only (IO), and SARS-CoV-2 infection after vaccine (IAV). Anti-SARS-CoV-2 spike protein (Anti-S) antibodies were measured by Elecsys Anti–SARS–CoV–2 S ELISA kit. Memory B cells ($CD19^+CD27^+$), plasmablast B cells ($CD19^+CD138^+$) and long-lived plasma cells (LLPC; $CD138^+CD19^-$) were measured by flow cytometry in 74 patients. Interferon gamma (IFN-γ) release by natural killer (NK) cells were measured by NKVue Test (NKMAX, Republic of Korea) in 76 patients. RT-PCR was performed with Bio-speedy® COVID-19 qPCR detection kit, Version 2 (Bioexen LTD, Istanbul, Turkey).
Results: The Anti-S antibodies were detectable in all HCWs (n: 224). The median Anti-S titers (BAU/mL) was significantly higher in VAI (620 25–75% 373–1341) compared to VO (136, 25–75% 85–283) and IO (111, 25–75% 54–413, $p < 0.01$). VAI group had significantly lower percentage of plasmablasts (2.9; 0–8.7) compared to VO (6.8; 3.5–12.0) and IO (9.9; 4.7–47.5, $p < 0.01$) (n:74). Percentage of LLPCs in groups VO, VAI and IO was similar. There was no difference of IFN-γ levels between the study groups (n: 76).
Conclusion: The antibody response was similar between uninfected vaccinated HCWs and unvaccinated HCWs who had natural infection. HCWs who had two doses of CoronaVac either before or after the natural SARS-CoV-2 infection elicited significantly higher antibody responses compared to uninfected vaccinated HCWs. The lower percentages of plasmablasts in the VAI group may indicate their migration to lymph nodes and initiation of the germinal center reaction phase. IFN-γ response did not differ among the groups.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak caused by a novel human coronavirus had started in late December 2019, which has later turned into a global pandemic [1]. Despite several public health precautions, the outbreak is still ongoing. As there are no antivirals to treat the SARS-CoV-2 infection, fast

* Corresponding author at: Fevzi Cakmak Mah Muhsinyazicioglu Cad No: 10 Marmara University Pendik Hospital, 34899 Istanbul, Turkey.
E-mail addresses: huseyin.bilgin@marmara.edu.tr (H. Bilgin), goncahaklar@marmara.edu.tr (G. Haklar), ondersirikci@marmara.edu.tr (O. Sirikci), emel.demiralp@memorial.com.tr (E. Eksioglu Demiralp).
[1] These authors contributed equally as second authors.
[2] Joined senior authors.

https://doi.org/10.1016/j.vaccine.2022.03.001
0264-410X/

introduction of newly developed vaccines against SARS-CoV-2 has been the main global strategy to control the pandemic.

Turkey has started mass vaccination with an inactivated whole virion vaccine, CoronaVac (Sinovac Life Science Co, Ltd, Beijing, China) in January 2021 and priority was given to healthcare workers (HCWs). In this program, two doses of CoronaVac were administered intramuscularly 28 days apart, including individuals who previously had had SARS-CoV-2infection [2]. According to the European Centre for Disease Prevention and Control report; as of 11 June 2021, a total of 333,678,903 COVID-19 vaccine doses had been distributed by manufacturers to European Union/European Economic Area (EU/EEA) countries. The number of HCWs is 1.061.035 in Turkey and vaccination priority was given to HCWs in January by administering 2 doses of inactivated SARS-CoV-2 vaccine one month apart (CoronaVac). A study from Turkey reported reduced crude mortality rates among HCWs after CoronaVac (between 2021 April 1 – May 17) compared to the pre-vaccination period (between 2020 March-2021 January) [3].

Although published studies were limited about the efficacy of inactivated whole virion vaccines, numerous publications about mRNA vaccines emerged fast. Human body generates antibodies directed against the spike protein of the virus (Anti-S) following SARS-CoV-2 infection, which declines months after the infection [4,5]. BNT162b2 mRNA COVID-19 (Pfizer/BioNTech) vaccine trials have shown 95% efficacy in preventing symptomatic infection in individuals without previous SARS-CoV-2 infection [6-9]. Trials with existing vaccines have shown varying degrees of humoral and cellular immune responses against SARS-CoV-2 [10-16]. A recent study done among fully vaccinated HCWs showed that humoral immune response decreases substantially in six months after the administration of the second dose of BNT162b2 vaccine [17]. It was also shown that in patients with previous SARS-CoV-2 infection, the antibody responses were increased after BNT162b2 mRNA COVID-19 (Pfizer/BioNTech) and mRNA-1273 (Moderna) vaccines [18,19]. The phase 3 trial results of CoronaVac from Turkey showed anti-receptor-binding domain (RBD) antibodies in 89.7% of vaccinated individuals' serum samples taken 14 days after the second dose [16]. There is very limited data about immune responses to CoronaVac, and antibody responses to vaccination after previous natural infection [20–22]. One study from Turkey had showed augmented antibody response to CoronaVac in individuals with previous SARS-CoV-2 infection [23].

There are studies investigating the peripheral blood lymphocyte profiles and bone marrow cell response to infection. SARS-CoV-2 spike protein-binding memory B cells were present at significantly higher frequencies than healthy controls at least 7 months after the onset of symptoms [6]. Adequate serum antibody titers are maintained by long-lived plasma cells (LLPC), which are non-replicating, antigen-specific plasma cells that are detected in the bone marrow long after the antigen is cleared [24].

NK cell driven interferon response and its effect on prognosis of SARS-CoV-2 infection has also been investigated. Studies have showed that patients with critical or severe infection had lower type I interferon (IFN) response [25]. On the other hand, increased IFN response have also been reported in patients with severe infection [26]. It has also been reported that measuring IFN response could be an indicator of the activation of innate immune response [27].

We aimed to determine 1) the humoral antibody response in HCWs who were vaccinated with CoronaVac, HCWs who had natural infection and compare the magnitude of the humoral antibody response of HCWs who were vaccinated and had COVID-19 infection, 2) to determine whether vaccination and/or natural infection have any effect on B cell phenotype, and natural killer (NK) cell response parameters 3) to describe the frequency of adverse events in vaccinated HCWs.

2. Methods

2.1. Study setting

The study was conducted between March – June 2021 in Marmara University Pendik E&R Hospital, Istanbul, Turkey on volunteering HCWs. The study was approved by the ethical committee of Marmara University (09.2020.740) and Turkish Ministry of Health. All participants have provided written informed consent. Investigators collected demographic information and adverse events occurring within 14 days after vaccination.

The participants were divided into four groups as shown below and blood samples were drawn to determine the anti-S antibodies, immunophenotyping and NK activation by IFN- γ.

- Vaccine only (VO); HCWs without documented SARS-CoV-2 infection and had received two doses of CoronaVac
- Vaccine after SARS-CoV-2 infection (VAI); HCWs who had had confirmed SARS-CoV-2 infection previously and had received two doses of CoronaVac afterwards
- SARS-CoV-2 infection only (IO); HCWs who had had confirmed SARS-CoV-2 infection previously and had not received CoronaVac
- SARS-CoV-2 infection after vaccine (IAV); HCWs who had received two doses of CoronaVac and later had confirmed SARS-CoV-2 infection ($\geq$2 weeks after the second dose of vaccination).

The adverse effects that might have occurred during the 14 days following each dose of vaccination was investigated by a questionnaire inquiring fever, myalgia, runny nose, fatigue, sore throat, rash, shortness of breath or other symptoms at the time blood samples were drawn. In addition, more serious adverse effects such as anaphylaxis and allergies occuring within a few hours after each dose of vaccination was sought via the hospital information system.

The groups were matched for age, gender, time after the SARS-CoV-2 infection and/or time after second dose of vaccine to blood sampling, except for IAV group, who were included later.

3. Laboratory studies

3.1. Antibody response

Antibodies (predominantly IgG, but also IgA and IgM) against the receptor binding domain (RBD) of the S protein of the SARS–CoV–2 virus were determined quantitatively in subjects' sera with a double-antigen sandwich immunoassay with electrochemiluminescence detection (Elecsys Anti–SARS–CoV–2 S kit, Cobas-e 601 analyzer, Roche Diagnostics, Basel, Switzerland). The results were obtained as U/mL. Since the Roche Elecsys Anti - SARS-CoV-2 S units per mL and WHO International Standards for anti-SARS-CoV-2 immunoglobulins were closely correlated (r^2 = 0.9992, slope = 0.972, intercept = 0.0072), the results were converted to BAU/mL (binding antibody units/mL) of the first WHO International Standard for anti-SARS-CoV-2 immunoglobulin [28,29].

3.2. Isolation of white blood cells (WBC) and B cell immunophenotyping

Peripheral blood WBCs were isolated from whole blood samples by using erythrocyte lysing solution. The following combinations with fluorochrome labelled monoclonal antibodies (mAb) and isotype-matched controls were used for three colors phenotypic analysis: 1) CD45-FITC / CD138-PE / CD19-PerCP-Cy.5.5; 2)

CD45-FITC / CD27-PE / CD19-PerCP-Cy.5.5 (Becton Dickinson Inc, San Jose, CA, USA). Following the incubation of $5x10^5$ cells per tube with adequate amount of antibodies as recommended by manufacturer for 20 minutes at room temperature in the dark, cells were washed and were immediately acquired and then analyzed using Diva software on a FACSCanto II (Becton Dickinson Inc, San Jose, CA, USA). Debris was excluded by using a gate that included all WBCs in the forward and side scatter plot or by using CD45/SSC plot. Lymphocytes were gated according to their forward and side scatter characteristics. $CD138^+CD19^+$ (plasmocytoid B cells); $CD138^+CD19^-$ (long lived plasma cells, LLPC); $CD27^+CD19^+$ (memory B cells) populations were evaluated as percentages.

3.3. NK activation by IFN-γ measurement

IFN-γ secretion by NK cells upon overnight activation by a specific NK activator was determined (NKVue Test, NKMAX, Republic of Korea). Briefly, peripheral venous blood samples were taken into special tubes which include NK stimulator (patented product) provided by the manufacturer. Tubes were directly incubated at 37 °C, 5% in a humidified CO_2 incubator during night. Next day, tubes were centrifuged at 2000 rpm for 15 minutes. Then, IFN-γ levels were measured in the supernatants of the tubes by using an IFN-γ ELISA kit provided by the manufacturer. The reference values for the NKVue test are > 500 pg/mL for normal, between 200 and 500 pg/mL for borderline and < 200 pg/mL for low IFN-γ response.

3.4. RT_PCR for SARS-CoV-2

Viral RNA was extracted from respiratory samples by using Bio-speedy® viral nucleic acid buffer (Bioexen LTD, Istanbul, Turkey) and RT-PCR was performed with Bio-speedy® SARS CoV-2 RT- qPCR detection kit, Version 2 (Bioexen LTD, Istanbul, Turkey) using primers and probes targeting the nucleocapsid (N) and ORF-1ab gene regions found in all SARS-CoV-2 and Human RNase P gene for the routine screening in a Biorad CFX-96 System (Biorad Laboratories INC., California, United States) B.1.1.7 detection for SARS CoV-2B.1.1.7 (UK) variant: Bio-speedy® SARS CoV-2 + VOC202012/01 RT-qPCR kit V1.0 (Bioexen LTD, Istanbul, Turkey) were used.

3.5. Statistics:

Descriptive analyses were presented as frequency and percentages, continuous values were expressed as [median (25th-75th percentiles)]. Categorical variables were compared with chi-square or Fisher's exact test. Paired proportions were compared with McNemar's test. Continuous variables between groups were compared with Mann-Whitney *U* test.

The VAI group would have higher antibody levels when compared to VO and IO groups was the main hypothesis of the study. Although there is a fourth group (IAV), this group was not included in the main hypothesis testing since the time elapsed from vaccination to blood collection was not comparable to other groups. A secondary analysis was done comparing the VAI and IAV groups. The analyses were performed with SPSS software version 23.0.

4. Results

A total of 224 HCWs were included in the study. The characteristics of the study groups are presented in Table 1. The median age was 31 (28–40) years and 56.4 % were female. The gender and age distribution were similar among groups. At least one comorbidity was present in 59 participants (26.3%). The most common comorbidity was asthma.

The median days from the second dose of vaccine to blood collection were 46 (41–47), 44 (42–46), and 109 (104–109) in groups VO, VAI, and IAV, respectively. The median days from COVID-19 diagnosis was 167 (135–333), 162 (125–296) and 52 (48–59) in groups VAI, IO, and IAV, respectively. A detailed explanation of intervals from infection and vaccination to blood collection is presented in Fig. 1.

The most common adverse events were fatigue (22/164), myalgia (14/164), and pain at injection site (10/164). The participants declared that the adverse events regressed within 48 h. Analysis of adverse events revealed that there was no significant difference among groups. VO group reported 23/75 (30.7%), VAI group had 18/53 (34.0%) and IAV group had 12/36 (33.3%) adverse events following any of the doses. Pairwise comparison showed significant decrease of adverse event frequency after the second dose (25.6% vs 17.7%, p = 0.04). The most common adverse events observed and the severity of all were similar to the findings in clinical phase trials.

4.1. Antibody response

Anti-S antibody concentrations (BAU/mL) of VO (136; 85–283) and IO (111; 54–413) groups were comparably elevated and were not significantly different (p = 0.62) from each other, whereas, Anti-S antibodies were significantly higher in VAI group (620; 373–1341) compared to VO and IO groups (p < 0.01). Strikingly, the IAV group had the highest antibody levels (6146; 2426–11137), but this data was not compared statistically because the duration from the 2nd dose of vaccine to blood sampling did not match other groups' intervals (Fig. 2A).

4.2. Memory B cells, plasmablasts and long-lived plasma cells

B Lymphocyte percentages, memory B cells ($CD19^+CD27^+$), plasmablasts ($CD19^+CD138^+$) and LLPC ($CD138^+CD19^-$) were evaluated in a total of 74 patients across groups (27 in VO; 24 in VAI; 23 in IO). No difference was observed in the percentage of B cells ($CD19^+$) among VAI (7.5; 0–12.4), VO (8.5; 7–12.0), and IO (9.3;7.3–12.0) groups (Fig. 2B).

VAI group had significantly lower percentage of $CD19^+138^+$ (2.9; 0–8.7) compared to VO (6.8; 3.5–12.0) and IO (9.9; 4.7–47.5) (Figure-2C) and lower percentage of $CD19^+27^+$ cells (13.5; 0–22.5) compared to VO (19.2; 15.0–26.6) and IO (18.9; 8.0–24.0) (p < 0.01) (Fig. 2D). There was no difference in the percentage of LLPC among groups (1.2 (0.7–1.7) in VO, 1.5 (07.-2.5) in IO and, 1.0 (0.2–2.1) in VAI groups) (Fig. 2E).

4.3. IFN- γ release by NK cells

NK cell response measured by IFN- γ release (pg/mL) upon stimulation was analyzed in 76 patients (9 in VO, 18 in VAI, 26 in IO and 23 in IAV group). The levels of interferon releasing response of NK cells of VO (276; 133–717), IO (412; 160–819), and VAI (414; 130–1105) groups were not different (Fig. 2F).

5. Discussion

We evaluated the anti-S antibody response in volunteering HCWs who were vaccinated with CoronaVac and had not documented SARS-CoV-2 infection until the time of blood sampling, HCWs who had had natural SARS-CoV-2 infection history before or after the vaccination and also HCWs who had natural SARS-CoV-2 infection history but were not vaccinated at the time of sampling in this observational, single-center case-control study. The anti-S antibody results in the VO group showed that the antibody

Table 1
Descriptive characteristics of the study groups.

	VO (n = 75)	VAI (n = 53)	IO (n = 60)	IAV (n = 36)
Age, median, (%25–75)	35 (28–42)	31 (29–41)	31 (27–40)	30 (26–39)
Gender, female, n (%)	43 (57.3)	30 (56.6)	34 (48.6)	25 (69.4)
Body mass index	24 (22–27)	25 (22–28)	25 (23–28)	24 (22–27)
Comorbidity present, n(%)	16 (21.3)	20 (37.7)	14 (20.3)	9 (25.0)
Days from COVID-19 diagnosis to blood collection	NA	167 (135–333)	162 (125–296)	52 (48–59)
Days from second dose of vaccination to blood collection	46 (41–47)	44 (42–46)	NA	109 (104–109)
Days from COVID-19 diagnosis to second dose of vaccination		94 (62–261)		55 (45–59)
Hospitalized, n (%)	NA	8 (15.1)	3 (5.0)	0
Comorbidity, n (%)	16 (21.3)	20 (37.7)	14 (23.7)	9 (25.0)
Asthma	4 (5.3)	2 (3.8)	2 (3.3)	4 (11.1)
Thyroid disorders	2 (2.7)	3 (5.7)	4 (6.7)	1 (2.8)
Allergic rhinitis	0	7 (13.2)	1 (1.7)	1 (2.8)
Hypertension	2 (2.7)	3 (5.7)	1 (1.7)	1 (2.8)
Diabetes	1 (1.3)	2 (3.8)	0	1 (2.8)
Malignancy	2 (2.7)	1 (1.9)	1 (1.7)	0
Rheumatologic	0	3 (5.7)	0	1 (2.8)
Miscellaneous	9 (12.0)	0	5 (8.3)	1 (2.8)
Adverse events				
Any adverse event after first or second dose	23 (30.7)	18 (34.0)	–	12 (33.3)
Fatigue	14 (18.7)	1 (1.9)	–	7 (19.4)
Myalgia	8 (10.7)	2 (3.8)	–	4 (11.1)
Pain at injection site	4 (5.3)	3 (5.7)	–	3 (8.3)
Sore throat	4 (5.3)	0	–	1 (2.8)
Fever	0	1 (1.9)	–	2 (5.6)
Dyspnea	1 (1.3)	1 (1.9)	–	1 (2.8)
Rash	0	1 (1.9)	–	1 (2.8)
Other	7 (9.3)	9 (17.0)	–	3 (8.3)

Abbreviations: VO; Vaccine Only, VAI; Vaccine After Infection, IO; Infection Only, IAV; Infection After Vaccination, NA; not applicable.

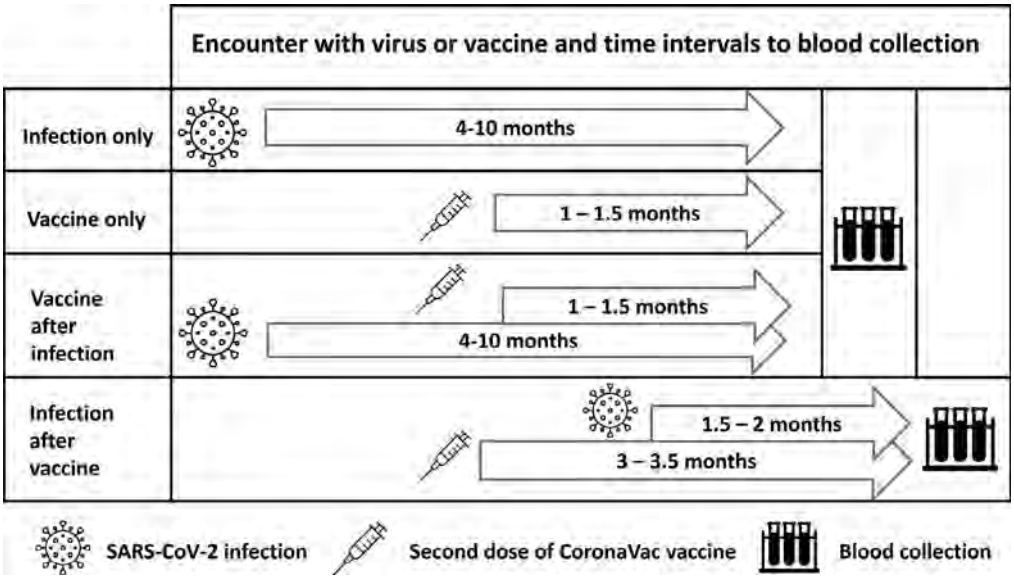


Fig. 1. Diagram of intervals from infection and vaccination to blood collection.

response elicited with CoronaVac 4–6 weeks after the 2nd dose of vaccination was comparable to the antibody concentrations obtained after natural infection in 4–10 months' time frame (25–75 percentile) in our IO cohort. Rus et al. had determined that a cut off of 133 BAU/mL for the Elecsys Anti–SARS–CoV–2 S kit to predict the presence of neutralizing antibodies [30]. In a recent article, one of the conclusions Gilbert et.al have reached was that the subgroups with neutralization titer 10 IU50/ml or with anti-spike IgG 33 BAU/ml, have about 75–85% reduction in COVID-19 risk compared to being unvaccinated [31]. Bergwerk et al. have showed that the neutralizing antibody titers were also correlated with IgG antibody titers [32], and higher antibody titers can provide better neutralizing activity which in the end results in better protection against infection [33,34]. Both the VO and the IO groups' anti-S antibody levels were high enough to assume the presence of neutralizing antibodies in our cohorts.

The VAI group, whose infection to sampling and vaccination to sampling times were matched to IO and VO groups respectively, had significantly higher anti-S antibody response, with a median of approximately 4–5 times than of IO and VO groups'. Similarly, Buonfrate et al had studied anti spike and anti- RBD IgG in 1935 HCWs and had reported that median antibody levels were significantly higher in individuals with past SARS-CoV-2 infection and were later vaccinated with Pfizer/Biontech [35]. Soysal et al. had

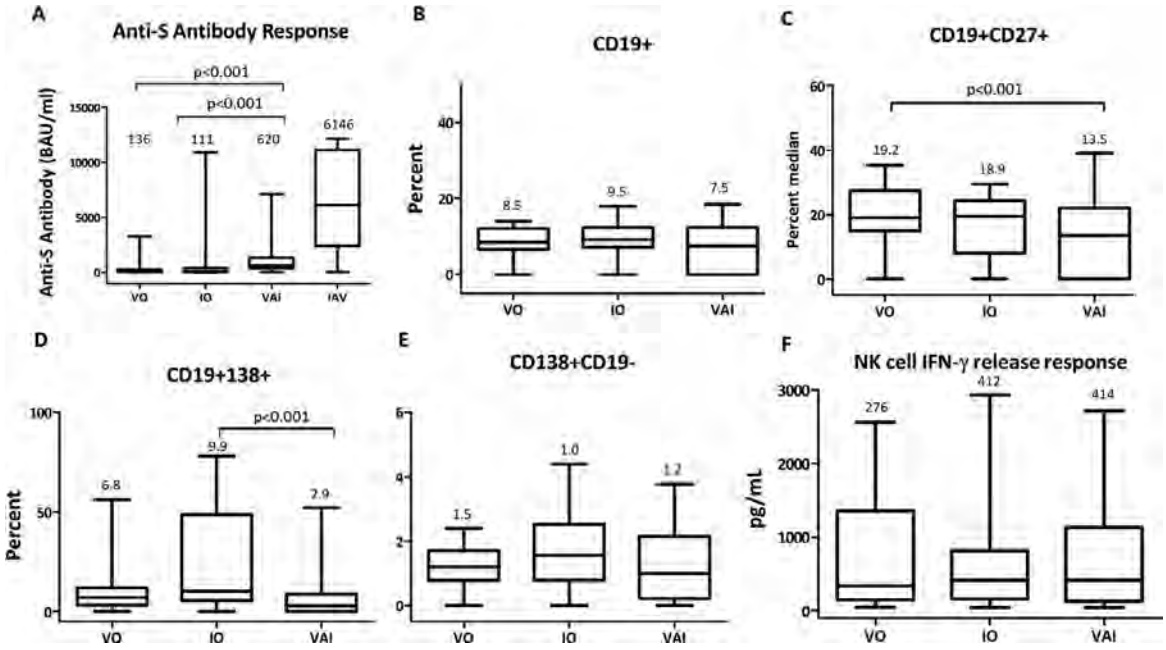


Fig. 2. Anti-S antibody concentrations (A), B cell percentages (B-E), and IFN- γ levels (F) measured in VO, IO, and VAI groups. Median values are noted above the box&whiskers plots. *VO: Vaccine only, IO: infection only, VAI: infection after vaccination, IAV: infection after vaccination, NK: Natural killer,* IFN- γ*: interferon gamma.*

investigated the immunogenicity of the CoronaVac in previously naturally infected HCWs and in line with our results, they showed that median antibody titers were significantly higher in previously infected HCWs (1220 AU/mL, range: 202–10328 AU/mL) compared to uninfected HCWs (913 AU/ml, range: 2.8–15547 AU/mL, p = 0.032). Yalçın et al, also has reported one log higher anti spike IgG levels in previously infected HCW's after single dose of CoronaVac. The antibody levels were higher 28 days after the second dose of CoronaVac in previously infected HCWs compared to uninfected HCWs (mean 1280 AU/mL vs 899 AU/mL, p < 0.001) [36]. The high antibody levels in VAI group suggests that the CoronaVac can boost the antibody response primed by natural infection. Our finding was in-line with these researchers' data showing that vaccination with either Pfizer/Biontech or CoronaVac vaccinations after natural infection exhibited a powerful booster effect and produced a significantly increased antibody response, which may be critical because Bergwerk et al have showed that HCWs with lower antibody titers are prone to re-infection with SARS-CoV-2 [32]. In several studies, previously infected HCWs elicited a higher antibody response compared to uninfected vaccinated and naturally infected individuals. Whether this difference translates to higher protection from infection needs further evaluation [9,37,38].

We observed that antibody levels of IAV group 6–8 weeks after the infection and 12 weeks after the vaccine was more than one log higher compared to VAI group (Fig. 2A). These two groups both had three encounters with the virus, either as vaccine or as natural infection. This group's data couldn't be statistically compared, since the times from the 2nd dose of vaccine to blood sampling were not matching other groups' intervals, but nevertheless, the difference among levels were striking. The antibody response that increases rapidly a month after natural infection (or vaccination, similarly) starts to decrease slowly in the following months. The interval between first encounter with the virus (vaccination in IAV group) and blood sampling was much shorter than the VAI groups, which might at least partly be the cause for the high antibody concentrations in IAV group [17]. These findings suggest that encountering with the virus for a third time boosts the antibody response and the level of antibody response may depend on the interval between encounters.

We can suggest that either before or after infection, or without an encounter with Sars Cov−2, CoronaVac vaccine can induce considerable amount of antibody response. This data suggests that booster shots may not be necessary or can be delayed in people who are infected and vaccinated. The relatively lower antibody levels in IO and VO groups may suggest that priority should be given to those groups for a third booster dose. However, third booster administration decision for such patients should depend on robust controlled trial results [39].

Ekşioğlu-Demiralp et al. had previously shown that B lymphocyte counts and percentages were decreased in patients who had active COVID-19 [40]. We found that, unlike active infection, B lymphocyte ($CD19^+$) percentages were all within normal limits with no difference among groups. This shows that the changes observed in B lymphocyte counts are a result of disease pathology, and the immunity acquired via natural infection or vaccination do not trigger related processes unless there is active disease.

One important marker in the assessment of humoral immunity evoked by either natural infections or through vaccination is the memory B ($CD19^+CD27^+$) lymphocytes [41]. The percentage of memory B cells evoked through vaccination (VO) or natural infection (IO) did not differ, and although the percentages observed in VAI group was found to be statistically lower than the VO group, all three groups were within refence ranges [42]. Since all three groups were within normal limits and the distribution of data in VAI group was wider, we did not attribute a clinical significance to the difference in the VAI group.

The plasmablasts as plasma cell precursors, and LLPC are responsible for antibody production. We observed the presence of plasmablasts ($CD19^+CD138^+$) in the peripheral blood in IO, VO, and VAI groups. The plasmablast percentages were comparable in IO and VO groups, but significantly decreased in the VAI group. The lower percentages of memory B cells and plasmablasts in the VAI group suggest that these cells might have migrated to lymph nodes from peripheral blood and have entered the germinal center

reaction phase. Germinal center reaction is a very important process in humoral immunity where the proliferation of B lymphocytes and affinity maturation of immunoglobulins occur. The high antibody production in the VAI group supports this hypothesis [43].

Significantly higher percentage of plasmablasts in IO group is noticeable. There are studies which report that increased plasmablasts may be responsible from autoantibody production [44,45]. The observance of autoantibodies in patients after COVID-19 have also shown to be associated with increased plasmablasts [46]. Therefore, the increase seen in IO group without an accompanying increase in anti S antibodies may be related with autoantibody production.

It was shown that SARS-CoV-2 infection had induced LLPC's in a recent article, by enriching bone marrow derived plasma cells and testing their anti-S antibody production response [6]. We also detected the presence of LLPC's in the peripheral blood in IO, VO and VAI groups, with no significant difference among groups. The presence of LLPC's might be an indication of a sustained adaptive activation following at least 6 weeks after vaccination or natural infection, and the presence of a similar response in IO and VO groups and a more than 5-fold response in VAI group shows that SARS Cov-2 specific LLPC's have been induced.

We found that IFN-γ release by NK cells upon stimulation was not different in all three groups, although the IFN-γ release data in each group was widely dispersed with the presence of very high and very low responders. NK activation is critical for viral defense due to their cellular cytotoxic function and their cytokine release in particular IFN-γ that activates both B and T cells [47]. It had been suggested that during COVID-19**,** SARS-COV2 was inhibiting the interferon signaling pathway [48]. Since we measured normal IFN-γ levels in all groups at least one month after infection, we might think that inhibitory effect of SARS-Cov2 on interferon secretion may be short lived, maybe encompassing only the active infection period.

5.1. Adverse effects

The frequencies of adverse effects reported after vaccinations were higher than the phase 3 clinical trials in Turkey (18.9%) [16], but were similar to the findings of another study in Turkey [49] (37.2% and 28.7%). The adverse event rates in VO, IAV and VAI groups were 30.7%, 33.3% and34.0%, respectively. Soysal et al. also reported that frequency of adverse events did not differ between the previously infected (35%) and uninfected (34%) groups [23]. The present study found similar adverse event rates without significant difference between the infected and uninfected groups. We also showed that the reporting of adverse reactions was significantly lower after the second dose of vaccination.

5.2. Strengths

To the best of our knowledge, this is the first study to investigate the humoral antibody response, B cell immunophenotype profile and NK cell response of individuals vaccinated with inactivated SARS-CoV-2 vaccine (CoronaVac) alone and together with natural SARS-CoV-2 infection.

5.3. Limitations

This study was conducted in healthcare workers, who were relatively young and healthy. This limits the generalizability of the study results for high-risk populations. The small sample size and lack of prevaccination antibody titers of the participants are other limitations.

6. Conclusion

The antibody response in uninfected vaccinated HCWs and HCWs who were unvaccinated and had had natural infection is similar to each other and comparable to levels obtained where the presence of neutralizing antibodies were shown. HCWs who had two doses of CoronaVac after natural SARS-CoV-2 infection elicited significantly higher antibody response, up to five times of the VO and IO groups', proving the basis for the requirement of a third dose of vaccination. HCWs who had SARS-CoV-2 after two doses of vaccination had one log higher antibody response, all showing that a third encounter with the virus after immunization, boosted the antibody response significantly. All groups had similar B lymphocyte percentages but the lower plasmablast percentages in VAI group indicate the migration of antibody producing cells from plasma to lymph nodes. The presence of LLPCs in all groups with similar percentages is indicative of sustained adaptive activation after immunization modeled in our groups. Since none of the groups had active infection at the time of sampling, IFN-γ response did not differ among groups.

- Author contributions

LM, GH, OS, AKY, SSY, MM, HB and EED initiated and coordinated the research. LM, GH, OS, EED, AKY, HB, MM, SSY, planned and recruited the study. LM, OS, GH, EED, AKY, US, HB, SSY and MM: conception and design of the study. OS, GH, AKY, MM, SSY, RCS and BC: acquisition of data. HB: statistical analysis. LM, OS, EED, AKY, US, MM, RCS, BC, SSY and HB: interpretation of data, drafting the article and revising it critically for important intellectual content. LM, OS, EED, GH, AKY, US, HB: final approval of the version to be submitted.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Zhu NA, Zhang D, Wang W, Li X, Yang BO, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382 (8):727–33.
[2] The Turkish Ministry of Health Covid19 information page, https://hsgm.saglik.gov.tr/en [accessed 20 March 2022].
[3] Akpolat T, Uzun O. Reduced mortality rate after coronavac vaccine among healthcare workers. J Infect 2021;83(2):e20–1.
[4] Vanshylla K, Di Cristanziano V, Kleipass F, Dewald F, Schommers P, Gieselmann L, et al. Kinetics and correlates of the neutralizing antibody response to SARS-CoV-2 infection in humans. Cell Host Microbe 2021;29(6):917–929.e4.
[5] Dan JM, Mateus J, Kato Yu, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021;371(6529). https://doi.org/10.1126/science:abf4063.
[6] Turner JS, Kim W, Kalaidina E, Goss CW, Rauseo AM, Schmitz AJ, et al. SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans. Nature 2021;595(7867):421–5.
[7] Li K, Huang B, Wu M, Zhong A, Li Lu, Cai Y, et al. Dynamic changes in anti-SARS-CoV-2 antibodies during SARS-CoV-2 infection and recovery from COVID-19. Nat Commun 2020;11(1). https://doi.org/10.1038/s41467-020-19943-y.
[8] Gerhards C, Thiaucourt M, Kittel M, Becker C, Ast V, Hetjens M, et al. Longitudinal assessment of anti-SARS-CoV-2 antibody dynamics and clinical features following convalescence from a COVID-19 infection. Int J Infect Dis 2021;107:221–7.
[9] L'Huillier AG, Meyer B, Andrey DO, Arm-Vernez I, Baggio S, Didierlaurent A, et al. Antibody persistence in the first 6 months following SARS-CoV-2 infection among hospital workers: a prospective longitudinal study. Clinical Microbiology and Infection 2021;27:784. e1-784. e8. https://doi.org/10.1016/j.cmi.2021.01.005
[10] Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med 2020;383 (27):2603–15.
[11] Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021;21(2):181–92.

[12] Walsh EE, Frenck RW, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. N Engl J Med 2020;383(25):2439–50.
[13] Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. The Lancet 2021;397(10269):99–111.
[14] Chen Y, Shen H, Huang R, Tong X, Wu C. Serum neutralising activity against SARS-CoV-2 variants elicited by CoronaVac. Lancet Infect Dis 2021;21 (8):1071–2.
[15] Sahin U, Muik A, Vogler I, Derhovanessian E, Kranz LM, Vormehr M, et al. BNT162b2 induces SARS-CoV-2-neutralising antibodies and T cells in humans. MedRxiv 2020.
[16] Tanriover MD, Doğanay HL, Akova M, Güner HR, Azap A, Akhan S, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. The Lancet 2021.
[17] Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months. N Engl J Med 2021;385(24):e84. https://doi.org/10.1056/NEJMoa2114583.
[18] Krammer F, Srivastava K, Alshammary H, Amoako AA, Awawda MH, Beach KF, et al. Antibody responses in seropositive persons after a single dose of SARS-CoV-2 mRNA vaccine. N Engl J Med 2021;384(14):1372–4.
[19] Manisty C, Otter AD, Treibel TA, McKnight Á, Altmann DM, Brooks T, et al. Antibody response to first BNT162b2 dose in previously SARS-CoV-2-infected individuals. Lancet 2021;397(10279):1057–8.
[20] Muena NA, García-Salum T, Pardo-Roa C, Serrano EF, Levican J, Avendaño MJ, et al. Long-lasting neutralizing antibody responses in SARS-CoV-2 seropositive individuals are robustly boosted by immunization with the CoronaVac and BNT162b2 vaccines. MedRxiv 2021.
[21] Bayram A, Demirbakan H, Günel Karadeniz P, Erdoğan M, Koçer I. Quantitation of antibodies against SARS-CoV-2 spike protein after two doses of CoronaVac in health care workers. J Med Virol 2021;93(9):5560–7.
[22] Seyahi E, Bakhdiyarli G, Oztas M, Kuskucu MA, Tok Y, Sut N, et al. Antibody response to inactivated COVID-19 vaccine (CoronaVac) in immune-mediated diseases: a controlled study among hospital workers and elderly. Rheumatol Int 2021;41(8):1429–40.
[23] Soysal A, Gönüllü E, Karabayır N, Alan S, Atıcı S, Yıldız İ, et al. Comparison of immunogenicity and reactogenicity of inactivated SARS-CoV-2 vaccine (CoronaVac) in previously SARS-CoV-2 infected and uninfected health care workers. Human Vacc Immunotherapeut 2021;17(11):3876–80.
[24] Halliley J, Tipton C, Liesveld J, Rosenberg A, Darce J, Gregoretti I, et al. Long-lived plasma cells are contained within the CD19− CD38hiCD138+ subset in human bone marrow. Immunity 2015;43(1):132–45.
[25] Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science 2020;369(6504):718–24.
[26] Wilk AJ, Rustagi A, Zhao NQ, Roque J, Martínez-Colón GJ, McKechnie JL, et al. A single-cell atlas of the peripheral immune response in patients with severe COVID-19. Nat Med 2020;26(7):1070–6.
[27] Boyarsky BJ, Werbel WA, Avery RK, Tobian AAR, Massie AB, Segev DL, et al. Immunogenicity of a single dose of SARS-CoV-2 messenger RNA vaccine in solid organ transplant recipients. JAMA 2021;325(17):1784. https://doi.org/10.1001/jama.2021.4385.
[28] Kristiansen PA, Page M, Bernasconi V, Mattiuzzo G, Dull P, Makar K, et al. WHO International Standard for anti-SARS-CoV-2 immunoglobulin. Lancet 2021;397 (10282):1347–8.
[29] Johum S, Degen H-J Correlation of the units (U) of the Elecsys® Anti-SARS-CoV-2 S assay to the "binding antibody units. of the first WHO International Standard for anti-SARS-CoV-2 immunoglobulin. Memo from Department of Research & Development for Centralized and Point of Care Solutions, Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg, Germany; 2021.
[30] Resman Rus K, Korva M, Knap N, Avšič Županc T, Poljak M. Performance of the rapid high-throughput automated electrochemiluminescence immunoassay targeting total antibodies to the SARS-CoV-2 spike protein receptor binding domain in comparison to the neutralization assay. J Clin Virol 2021;139:104820. https://doi.org/10.1016/j.jcv.2021.104820.
[31] Gilbert PB, Montefiori DC, McDermott AB, Fong Y, Benkeser D, Deng W, et al. Immune Assays Team § Moderna, Inc., Team § Coronavirus Vaccine Prevention Network (CoVPN)/Coronavirus Efficacy (COVE) Team § United States Government (USG)/CoVPN Biostatistics Team § Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. Science 2022;375 (6576):43–50.
[32] Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, et al. Covid-19 breakthrough infections in vaccinated health care workers. N Engl J Med 2021;385(16):1474–84.
[33] Criscuolo E, Diotti RA, Strollo M, Rolla S, Ambrosi A, Locatelli M, et al. Weak correlation between antibody titers and neutralizing activity in sera from SARS-CoV-2 infected subjects. J Med Virol 2021;93(4):2160–7.
[34] Lustig Y, Sapir E, Regev-Yochay G, Cohen C, Fluss R, Olmer L, et al. BNT162b2 COVID-19 vaccine and correlates of humoral immune responses and dynamics: a prospective, single-centre, longitudinal cohort study in health-care workers. The Lancet Respiratory Medicine 2021;9:999–1009. https://doi.org/10.1016/S2213-2600(21)00220-4.
[35] Buonfrate D, Piubelli C, Gobbi F, Martini D, Bertoli G, Ursini T, et al. Antibody response induced by the BNT162b2 mRNA COVID-19 vaccine in a cohort of health-care workers, with or without prior SARS-CoV-2 infection: a prospective study. Clin Microbiol Infect 2021;27(12):1845–50.
[36] Yalçın TY, Topçu Dİ, Doğan Ö, Aydın S, Sarı N, Erol Ç, et al. Immunogenicity After Two Doses of Inactivated Virus Vaccine in Healthcare Workers with and without Previous COVID-19 Infection: Prospective Observational Study. J Med Virol 2022;94(1):279–86.
[37] Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med 2021;27(7):1205–11.
[38] Levine-Tiefenbrun M, Yelin I, Katz R, Herzel E, Golan Z, Schreiber L, et al. Initial report of decreased SARS-CoV-2 viral load after inoculation with the BNT162b2 vaccine. Nat Med 2021;27(5):790–2.
[39] Krause PR, Fleming TR, Peto R, Longini IM, Figueroa JP, Sterne JAC, et al. Considerations in boosting COVID-19 vaccine immune responses. The Lancet 2021;398(10308):1377–80.
[40] Eksioglu-Demiralp E, Alan S, Sili U, Bakan D, Ocak I, Yurekli R, et al. Peripheral innate and adaptive immune cells during COVID-19: Functional neutrophils, pro-inflammatory monocytes and half-dead lymphocytes. MedRxiv 2020.
[41] Takemori T, Tarlinton D, Hiepe F, Andreas R. B cell memory and Plasma cell development. Molecular Biology of B cells: Academic Press; 2014. p. 227–49.
[42] Caraux A, Klein B, Paiva B, Bret C, Schmitz A, Fuhler GM, et al. Circulating human B and plasma cells. Age-associated changes in counts and detailed characterization of circulating normal CD138- and CD138+ plasma cells. Haematologica 2010;95(6):1016–20.
[43] Oropallo MA, Cerutti A. Germinal center reaction: antigen affinity and presentation explain it all. Trends Immunol 2014;35(7):287–9.
[44] Tipton CM, Fucile CF, Darce J, Chida A, Ichikawa T, Gregoretti I, et al. Diversity, cellular origin and autoreactivity of antibody-secreting cell population expansions in acute systemic lupus erythematosus. Nat Immunol 2015;16(7):755–65.
[45] Jenks SA, Cashman KS, Woodruff MC, Lee F-E-H, Sanz I. Extrafollicular responses in humans and SLE. Immunol Rev 2019;288:136–48.
[46] Schultheiß C, Paschold L, Willscher E, Simnica D, Wöstemeier A, Muscate F, et al. Maturation trajectories and transcriptional landscape of plasmablasts and autoreactive B cells in COVID-19. Iscience 2021:103325. https://doi.org/10.1016/j.isci.2021.103325.
[47] Paul WE. Fundamental Immunology. Fundamental Immunology. 7th ed., Wolters Kluwer Health/Lippincott Williams & Wilkins; 2013, p. 1283.
[48] Park A, Iwasaki A. Type I and type III interferons–induction, signaling, evasion, and application to combat COVID-19. Cell Host & Microbe 2020;27:870–8. https://doi.org/10.1016/j.chom.2020.05.008.
[49] Gümüş HH, Ödemiş İ, Alışka HE, Karslı A, Kara S, Özkale M, et al. Side effects and antibody response of an inactive severe acute respiratory syndrome coronavirus 2 vaccine among health care workers. Revista Da Associação Médica Brasileira 2021;67(12):1825–31.

1.4. CoronaVac preveniu Covid-19 em 93% dos profissionais de saúde na Turquia, mostra estudo

Um artigo publicado na revista Human Vaccines & Immunotherapeutics mostrou que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, teve uma efetividade de 93% para prevenir a Covid-19 em profissionais de saúde na Turquia, população com maior nível de exposição ao vírus SARS-CoV-2. O estudo foi conduzido pela Faculdade de Medicina da Universidade Yildirim Beyazit entre julho e agosto de 2021.

Participaram da pesquisa 627 profissionais de saúde do Hospital da Cidade de Ankara, sendo 158 homens e 469 mulheres com idade média de 35 anos. Destes, 536 foram imunizados com a CoronaVac e 91 não foram vacinados. Os voluntários responderam a um questionário sobre características demográficas, status de vacinação e histórico de Covid-19 antes ou após a imunização.

Dos indivíduos imunizados com a vacina do Butantan, apenas 38 (7%) desenvolveram Covid-19 após a vacinação, sem necessidade de internação, e 146 (27%) tiveram a doença antes de tomar o imunizante. Já entre os participantes não vacinados, cerca de 50% (46) contraíram a doença.

Um estudo clínico de fase 3 conduzido na Turquia já havia demonstrado uma eficácia de 83,5% da CoronaVac contra casos sintomáticos de Covid-19 e 100% contra a doença grave. Já uma pesquisa de efetividade conduzida no Chile mostrou que a vacina protegeu 65,9% contra casos sintomáticos, 87,5% contra hospitalizações e 90,3% contra internações.

Os cientistas turcos concluíram que, assim como foi observado em outros estudos, os seus resultados comprovam a eficácia e segurança do imunizante. "Considerando que aproximadamente 80% dos casos de Covid-19 em nossa análise ocorreram antes da vacinação do grupo imunizado, acreditamos que a CoronaVac é ainda mais eficaz em prevenir a doença do que os dados anteriores apontaram", afirmam.

Publicado em: 16/02/2022

Artigo

Human Vaccines & Immunotherapeutics

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/khvi20

Effectiveness of CoronaVac in preventing COVID-19 in healthcare workers

Gulsum Iclal Bayhan & Rahmet Guner

To cite this article: Gulsum Iclal Bayhan & Rahmet Guner (2022): Effectiveness of CoronaVac in preventing COVID-19 in healthcare workers, Human Vaccines & Immunotherapeutics, DOI: 10.1080/21645515.2021.2020017

To link to this article: https://doi.org/10.1080/21645515.2021.2020017

Published online: 16 Feb 2022.

Artigo

HUMAN VACCINES & IMMUNOTHERAPEUTICS
https://doi.org/10.1080/21645515.2021.2020017

Taylor & Francis
Taylor & Francis Group

RESEARCH PAPER

OPEN ACCESS Check for updates

Effectiveness of CoronaVac in preventing COVID-19 in healthcare workers

Gulsum Iclal Bayhan [a,b] and Rahmet Guner [b]

[a]Department of Pediatric Infectious Disease Ankara City Hospital, Children's Hospital, Faculty of Medicine, Yildirim Beyazit University, Ankara, Turkey; [b]Department of Infectious Disease and Clinical Microbiology, Ankara City Hospital, Faculty of Medicine, Yildirim Beyazit University, Ankara, Turkey

ABSTRACT

The CoronaVac vaccine was found to be effective against symptomatic COVID-19 and protective against severe disease in phase 3 studies. However, there are little data about its effectiveness in real-world conditions. The aim of the current study was to investigate the protective effect of the CoronaVac vaccine in health-care workers (HCWs) in Turkey, a country where CoronaVac is widely used. The questionnaire was sent to all employees in the form of a survey link by using a telephone application. In the survey, HCWs were asked about demographic characteristics; CoronaVac vaccination status, history of a COVID-19 infection, whether COVID-19 infection was before or after the CoronaVac vaccination; the time between being vaccinated and the COVID-19 infection; the clinical pictures of COVID-19 infection. Those who experienced COVID-19 before vaccination were compared with the breakthrough cases in terms of demographic and clinical features. A total of 628 HCW agreed to participate in the study. A total of 536 (85.3%) volunteers had been vaccinated and 92 (14.6%) had not been vaccinated against COVID-19 with CoronaVac. There was a history of COVID-19 infection in 234 (37.2%) subjects and 188 (35%) had been vaccinated and 46 (50%) not vaccinated. The rate experiencing COVID-19 disease was significantly lower in the vaccinated than the unvaccinated volunteers. The rate of breakthrough cases after CoronaVac was found to be 7%. The hospitalization rate was similar in the breakthrough cases and those who had COVID-19 before CoronaVac vaccination. The results of our study indicate that CoronaVac provides protection against COVID-19.

ARTICLE HISTORY
Received 16 September 2021
Revised 24 November 2021
Accepted 14 December 2021

KEYWORDS
CoronaVac; Sinovac; COVID-19; pneumonia; hospitalization; healthcare workers; mortality; survey; vaccine; intensive care unit

Introduction

The SARS-CoV-2 pandemic continues to wreak havoc around the world although 18 months have gone by since it first began. The pandemic has caused a large number of deaths worldwide and adversely affected how we live in many ways, with especially significant effects on the education system, the economy, and social life. The agent is highly contagious and has a wide range of clinical courses from asymptomatic to acute respiratory distress syndrome (ARDS), multi-organ failure, and death. Asymptomatic persons can also be contagious. The method thought to present the best hope to end the pandemic is vaccines and the discovery of vaccines against SARS-CoV-2 has opened a new era in the fight against the disease. The vaccines approved by the World Health Organization (WHO) by August 2021 were the BNT162b2 messenger RNA (mRNA) vaccine (Pfizer–BioNTech), the ChAdOx1 nCoV-19 vaccine (Oxford–AstraZeneca), the mRNA-1273 vaccine (Moderna), the Ad26.COV2.S vaccine (Johnson & Johnson), the inactivated SARS-CoV-2 vaccine (Vero cell) (Sinopharm/BIBP), and the inactivated SARS-CoV-2 vaccine (Vero cell) (Sinovac Life Sciences).[1]

Following the emergency use approval of the CoronaVac vaccine by the Turkish Ministry of Health on 13 January 2021, vaccination was started in Turkey on 14 January 2021. The first target group was health-care workers (HCW) and the vaccine was administered in two doses at a 28-day interval. Application of the BioNTech vaccine in Turkey started on 1 June 2021. The third dose of the CoronaVac or the BioNTech vaccine is currently being administered to health-care workers at the request of the person, starting at 5 months after the second dose.

The CoronaVac vaccine is an inactivated whole-virion vaccine. The World Health Organization (WHO) has approved the vaccine for emergency use on 1 June 2021.[1] The most important factor for choosing the CoronaVac vaccine when starting vaccination in Turkey was its ability to be stored without the need for freezing, in addition to the opinion that the more traditional technology using the whole virus that was employed for its manufacture would be more acceptable by the public. Side effects of CoronaVac are rare and those reported have generally been mild, the most common ones being fatigue, injection site pain, and sore muscles.[2]

The vaccine was found to be 50.7–83.5% effective against symptomatic COVID-19 and 100% protective against severe disease in phase 3 studies conducted in Brazil and Turkey.[2,3] However, there is little data about its effectiveness in real-world conditions. The SARS-CoV-2 antibody levels have been shown to decrease 5 months after vaccination in adults vaccinated with CoronaVac, just like the decrease in post-infection antibody levels but studies on how long vaccine protection lasts are inadequate.[4] There is also increasing concern about the vaccine's effectiveness against the new variants.

CONTACT Gulsum Iclal Bayhan gibayhan@ybu.edu.tr Department of Pediatric Infectious Disease, Ankara City Hospital, Children's Hospital, Universiteler mahallesi, Ankara, Turkey

The current study aimed to investigate the protective effect of the CoronaVac vaccine in HCWs in Turkey, a country where CoronaVac is widely used. Our study was carried out 7.5 months after the first vaccination with CoronaVac in Turkey and aims to provide information about the long-term protective effects of this vaccine.

Materials and methods

The study was conducted between 27 July and 16 August 2021, 7.5 months after COVID-19 vaccination was started, on HCWs working at Ankara City Hospital, a health campus with a capacity of 3810 beds and consisting of 8 separate hospitals. The questionnaire was sent to all employees in the form of a survey link by using a telephone application. In total, 628 healthcare workers volunteered to participate in the study. Each volunteer could complete the questionnaire only once.

The questionnaire consisted of 12 questions on the following topics: (1) demographic characteristics (age, gender, profession); (2) history of or current work in units that care for COVID-19 patients (inpatient service, intensive care, emergency room, outpatient clinic); (3) whether vaccinated with 2 doses of CoronaVac (Sinovac®); (4) whether a third dose of the COVID-19 vaccine had been administered; (5) which vaccine had been used for the third dose, if any (CoronaVac vs. BioNTech); (6) whether there was a history of a COVID-19 infection (confirmed by SARS-CoV-2 PCR); (7) whether this infection was before or after the CoronaVac vaccination; (8) if the infection was after the vaccination, whether it was after the 1st dose or the 2nd dose or the 3rd dose, if any; (9) the time between being vaccinated and the COVID-19 infection, if any, in the post-vaccine period; (10) which of the four clinical pictures of COVID-19 infection had been present (asymptomatic; only loss of taste and smell; flu-like picture with coryza and/or coughing and/or nasal discharge and/or bone-joint pain; physician-diagnosed lower respiratory tract infection; (11) history of hospitalization during the COVID-19 infection; (12) history of intensive care unit stay during the COVID-19 infection.

Cases with COVID-19 infection ≥14 days after the vaccination were considered breakthrough cases. Those who experienced COVID-19 before vaccination or those who were not vaccinated and experienced COVID-19 were compared with the breakthrough cases in terms of demographic and clinical features. The rate of past COVID-19 infection in subjects who were and were not vaccinated was also compared. Ethics committee approval was obtained for this study (Approval number E2-21-709).

Results

A total of 628 subjects, consisting of 158 (25.2%) males and 470 (74.8%) females, volunteered to complete the questionnaire. The mean age of the volunteers was 35.5 ± 8.9 years (4 subjects were aged ≥60 years). There were 432 (68.8%) subjects who had worked or continued to work in units that cared for COVID-19 patients. A total of 536 volunteers had been vaccinated and 92 (14.6%) had not been vaccinated against COVID-19 with CoronaVac. Of the 92 unvaccinated subjects, only one had been vaccinated with two doses of BioNTech in June 2021. This healthcare worker was excluded because she was fully vaccinated, and the total number of unvaccinated personnel was found to be 91 (14.5%). A third dose had been administered to 355 subjects; this was BioNTech in 302 (85.1%) and CoronaVac in 53 (14.9%) (Figure 1).

There were a history of COVID-19 infection in 234 (37.2%) subjects and 188 (35%) had been vaccinated and 46 (50%) not vaccinated. The rate experiencing COVID-19 disease was significantly lower in the vaccinated than the unvaccinated volunteers (p = .017). Among the subjects who had a history of COVID-19 disease and vaccination, 146 (77.7%) had the COVID-19 infection before the CoronaVac vaccination, and 42 (22.3%) afterward. Of the latter 42 subjects, 38 (90.2%) had

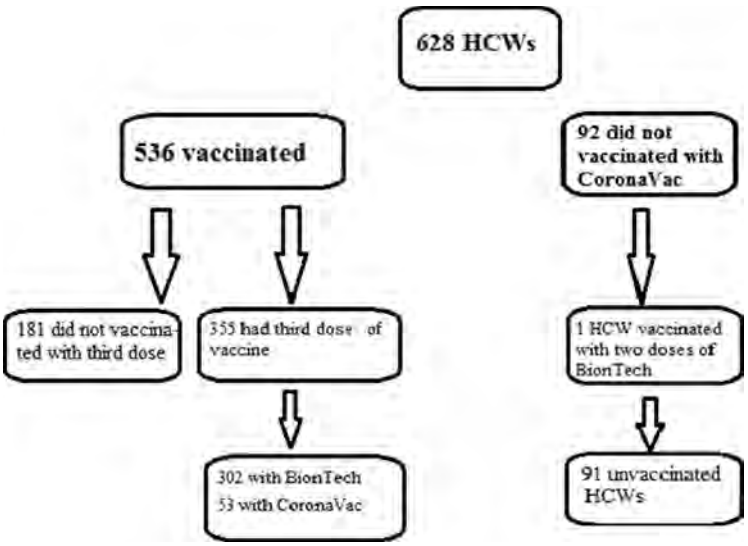


Figure 1. The vaccination status of health care workers.

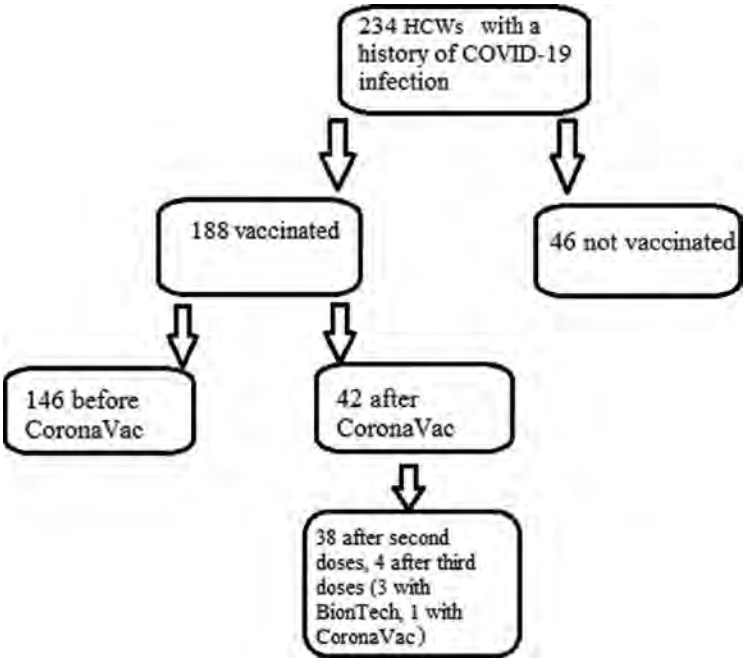


Figure 2. Vaccination history of HCWs who had experienced the COVID-19 infection.

experienced the COVID-19 infection after the second dose and 4 (9.8) after the third dose (Figure 2). In the group that experienced COVID-19 infection after the third dose, 3 (75%) were vaccinated with BioNTech and 1 with CoronaVac; of these four HCWs, 1 HCW had received the third dose of CoronaVac vaccine experienced COVID-19 infection one month after the last dose of CoronaVac. Among the other three HCWs who had received the BioNTech vaccine, one experienced COVID-19 7 days after the third dose while the other two had the disease one month afterward.

The median interval from the second vaccine dose to the COVID-19 diagnosis was two months (min. 7 days-max 6 months). One HCW had COVID-19 seven days after receiving second dose of the CoronaVac vaccine. After this subject and 3 subject who experienced COVID-19 after third doses of vaccination with BioNTech were excluded, the rate of breakthrough cases was found to be 7% (38/536). The comparison of the demographic and clinical characteristics of the HCWs with breakthrough infection and the HCWs with COVID-19 infection before vaccination or HCWs who were not vaccinated and experienced COVID-19 infection is shown in Table 1.

The rate of vaccination with the third dose lower in those who had COVID-19 after vaccination (12, 31.6%) than those who had COVID-19 before vaccination (83, 54.8%) (p = .008). None of the HCW with COVID-19 history in the study group required ICU admission during COVID-19. According to hospital records, a pharmacist working in our hospital died before the vaccination started, there were no other HCW who died.

Table 1. The comparison of HCWs who experienced COVID-19 after two doses of the CoronaVac vaccine with HCWs who did not vaccinated and experienced COVID-19 or experienced COVID-19 before two doses of the CoronaVac vaccine as regards the demographic features and COVID-19 clinical picture, hospitalization during COVID-19, and vaccination with the third dose.

	COVID-19 before vaccination or COVID-19 in unvaccinated n: 192	COVID-19 after vaccination n: 38	p
Age (mean ± SD)	34.8 ± 9.4	36.6 ± 7.4	.20
Gender (female percentage) n (%)	102 (69.8)	30 (78.9)	.34
Occupation n (%)			
• Doctor	70 (36.5)	13 (34.2)	.98
• Nurse-midwife-healthcare technician	51 (26.5)	10 (26.3)	
• Secretary, administration or maintenance worker, pharmacist, language therapist, child development specialist	71 (37.0)	15 (39.5)	
Working at COVID-19 clinics	138 (71.9)	27 (71)	.15
Clinical picture of COVID-19			
• Asymptomatic	18 (9.4)	4 (10.5)	.86
• Taste and smell loss only	23 (12)	6 (15.8)	
• Influenza-like illness	118 (61.5)	20 (52.6)	
• Pneumonia	33 (17.1)	8 (21.1)	
Hospitalization during COVID-19	20 (10.4)	4 (10.5	.93

Discussion

The results of our study indicate that CoronaVac provides protection against COVID-19. However, there was no difference in disease severity or hospitalization rate between those who had COVID-19 before or after vaccination.

The results of Phase 3 studies conducted in Turkey have shown vaccination with two doses of CoronaVac vaccine to reduce the risk of developing symptomatic COVID-19 in the 18–59 years age group by 83.5% and to prevent COVID-19-related hospitalization by 100% when compared to placebo.[2] In a Phase-3 study conducted in Brazil, the protection rate against symptomatic COVID-19 was found to be 50.7%, while protection against a severe clinical picture was 100%, similar to the Turkish study.[3] In a prospective observational cohort study involving approximately 10.2 million persons and conducted in Chile, the effectiveness was 65.9% in preventing COVID-19, 87.5% in preventing hospitalization, and 90.3% in preventing ICU admission.[5] The rate of being sick with COVID-19 was significantly lower in vaccinated HCWs than those who were not vaccinated in the current study. Considering that approximately 80% of the cases of COVID-19 were before CoronaVac vaccination in the vaccinated group in our study, we believe that CoronaVac is even more effective in preventing COVID-19 infection than the above numbers indicate. In our study, the median time between vaccination and COVID-19 infection was 2 months. This period also coincides with an increase in the number of cases in Turkey. In addition, after the two doses of CoronaVac vaccination, there was an increase in the behaviors of the health personnel to spend time together without the mask and social distance rules with the belief that those who have been vaccinated will no longer get COVID-19.

Some of the most important expectations from the COVI9-19 vaccines are reduced hospitalization rates and hospital occupancy rates to ensure the maintenance of the quality of service in the healthcare system. It has been reported that CoronaVac prevents hospitalization due to COVID-19 by 87.5% to 100%.[2,5] Approximately one-fifth of the breakthrough cases had been hospitalized in the current study, and the hospitalization rate was similar in the breakthrough cases and those who had COVID-19 before CoronaVac vaccination or those who were unvaccinated. The demographic features of those with unvaccinated/pre-vaccine and post-vaccine COVID-19 were also similar. We are unable to comment on the impact of co-morbidity on the hospitalization rate as it was not included in the questionnaire. In our county, when healthcare workers get COVID-19, decision of hospitalization can be made even if there is no indication for hospitalization, often to protect the household. This may be the reason for the high hospitalization rate in this study.

The most significant benefits of the CoronaVac vaccine are reported to be preventing a severe clinical picture and intensive care unit hospitalization.[3,5] None of the breakthrough cases or those who had COVID-19 before CoronaVac vaccination was admitted to intensive care in the current study. The absence of intensive care admissions in our study cohort may be due to the fact that it consisted of a relatively young age group with very few subjects aged >60 years. Another reason may be the low rate of subjects with co-morbid conditions, but we did not question the co-morbidity status and cannot reach a definite conclusion.

Breakthrough infections have been reported after other COVID-19 vaccines as well.[6] In a study conducted on HCWs, the rate of breakthrough cases among those vaccinated with BioNTech was 0.4%. The majority of breakthrough cases have been reported to be asymptomatic.[7] The rate of breakthrough cases in the current study was 7%, higher than the rate reported with the BioNTech vaccine, and most cases were symptomatic.

The emergence of new variants has resulted in contemplating whether the vaccines would be effective against these new variants as well. There are only a few studies on the effectiveness of the CoronaVac vaccine against the new variants. The P.1 lineage or Gamma variant virus has been shown to evade neutralizing antibodies induced by the inactivated SARS-CoV -2 vaccine.[4] In another study conducted in Brazil at a time when 86% of the genotypes were found to be of the gamma variant, being vaccinated with two doses of CoronaVac was 37.1% effective in preventing COVID-19.[8] While B.1.1.7 (Alpha) was previously the dominant variant in Turkey, the delta variant was first detected in April 2021 and became the predominant variant by August 2021.[9,10] The variant of SARS-CoV-2 that infected the HCWs was not investigated in the current study. Considering that the median time between vaccination and getting COVID-19 in the HCWs who stated that they were infected after the vaccination was 2 months, the delta variant was present in Turkey during that period. There is therefore a possibility that these individuals were infected with the delta variant. However, the date range of our study includes only a small part of the period in which the delta variant became the dominant variant in the community. The delta variant possesses enhanced infectivity and replication ability and it has been found to be associated with an increase in symptomatic breakthrough infections following mRNA vaccines. However, the upper respiratory tract SARS-CoV-2 IgG level has been found at low levels or to be absent in all subjects who had a breakthrough infection with the delta variant. Such infection is therefore believed to be the result of waning immunity in vaccinated individuals.[11,12] It was reported that neutralizing antibody titers against the delta variant in CoronaVac recipients were significantly lower than unvaccinated, naturally infected patients.[13]Additional studies are needed on breakthrough infections associated with the delta variant in subjects vaccinated with CoronaVac in countries where the delta variant has become predominant, as in Turkey.

The rate of vaccination with the third dose lower in those who had COVID-19 after vaccination than those who had COVID-19 before vaccination. This may be due to the awareness about protection from reinfection after recovery.[14] It has been reported that subjects who recovered and produced antibodies to SARS-CoV-2 were protected from reinfection for at least six months.

The strength of our study is that it was carried out in healthcare workers, who are at high risk of getting COVID-19 and who have a high probability of undergoing a PCR test when they suspect COVID-19 infection in themselves. In support of this notion, one-third of our study population had experienced COVID-19. A limitation of our study was that co-morbidity was not investigated and we were therefore unable to reveal whether the similarity of hospitalizations and

clinical presentations was due to a difference in the comorbidity distribution between the groups. Another limitation of our study is that it did not cover the period when the delta variant was predominant in Turkey. About half of our study group had been vaccinated with a single dose of BioNTech six months after CoronaVac vaccination. Our study period covered approximately 2 months after BioNTech vaccination. A third dose of vaccination with BioNTech possibly compensated for the decreased effectiveness of CoronaVac 6 months after vaccination. This was another limitation of our study.

In conclusion, CoronaVac is protective against SARS-CoV -2 infection. Further research is needed on the protection provided by CoronaVac in the elderly and those with comorbid conditions and against emerging variants, in addition to how long this protection lasts.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

ORCID

Gulsum Iclal Bayhan http://orcid.org/0000-0002-1423-4348
Rahmet Guner http://orcid.org/0000-0002-1029-1185

References

1. WHO. [accessed 2021 Sept 16]. https://extranet.who.int/pqweb/sites/default/files/documents/Status%20of%20COVID-19%20Vaccines%20within%20WHO%20EUL-PQ%20evaluation%20process%20-%203%20June%202021r.pdf .
2. Tanriover MD, Doğanay HL, Akova M, Güner HR, Azap A, Akhan S, Köse Ş, Erdinç FŞ, Akalın EH, Tabak ÖF, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. Lancet. 2021;398:213–22. doi:10.1016/S0140-6736(21)01429-X.
3. Palacios R, Batista AP, Albuquerque CSN, Patiño EG, Santos JP, Conde MTRP, Piorelli RO, Júnior LCP, Raboni SM, Ramos F, et al. Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in Brazil: the PROFISCOV study. SSRN. published online 2021 Apr 14. doi:10.2139/ssrn.3822780. (preprint).
4. Souza WM, Amorim MR, Sesti-Costa R, Coimbra LD, Brunetti NS, Toledo-Teixeira DA, de Souza GF, Muraro SP, Parise PL, Barbosa PP, et al. Neutralisation of SARS-CoV-2 lineage P.1 by antibodies elicited through natural SARS-CoV-2 infection or vaccination with an inactivated SARS-CoV-2 vaccine: an immunological study. Lancet Microbe. 2021 July 8;2:e527–e535. doi:10.1016/S2666-5247(21)00129-4.
5. Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, Pizarro A, Acevedo J, Leo K, Leon F, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. N Engl J Med 285 . 2021 :875–884.
6. Duerr R, Dimartino D, Marier C, Zappile P, Wang G, Lighter J, Elbel B, Troxel AB, Heguy A. Dominance of alpha and Iota variants in SARS-CoV-2 vaccine breakthrough infections in New York City. J Clin Invest. 2021 Aug 10;131:152702. doi:10.1172/JCI152702.
7. Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, Mandelboim M, Gal Levin E, Rubin C, Indenbaum V, et al. Covid-19 breakthrough infections in vaccinated health care workers. N Engl J Med. 2021 July 28;385:1474–84. doi:10.1056/NEJMoa2109072.
8. Hitchings MDT, Ranzani OT, Torres MSS, de Oliveira SB, Almiron M, Said R, Borg R, Schulz WL, de Oliveira RD, Da Silva PV, et al. Effectiveness of CoronaVac among healthcare workers in the setting of high SARS-CoV-2 Gamma variant transmission in Manaus, Brazil: a test-negative case-control study. Lancet Reg Health Am. 2021 Sept;1:100025. doi:10.1016/j.lana.2021.100025.
9. [accessed 2021 Sept 16]. https://www.dw.com/tr/delta-varyant%C4%B1-t%C3%BCrkiyede-ne-kadar-yayg%C4%B1n/a-57984629 .
10. [accessed 2021 Sept 16]. https://www.aa.com.tr/tr/koronavirus/koronavirus-bilim-kurulu-uyesi-korukluoglu-agustosta-vakalarin-yuzde-80i-delta-varyanti-olacak/2298422 .
11. Luo CH, Morris CP, Sachithanandham J, Amadi A, Gaston D, Li M, Swanson NJ, Schwartz M, Klein EY, Pekosz A, et al. Infection with the SARS-CoV-2 delta variant is associated with higher infectious virus loads compared to the alpha variant in both unvaccinated and vaccinated individuals. medRxiv. 2021 Aug 20;8(15):21262077. doi:10.1101/2021.08.15.21262077. (Preprint).
12. Brown CM, Vostok J, Johnson H, Burns M, Gharpure R, Sami S, Sabo RT, Hall N, Foreman A, Schubert PL, et al. Outbreak of SARS-CoV-2 infections, including COVID-19 vaccine breakthrough infections, associated with large public gatherings - Barnstable County, Massachusetts, July 2021. MMWR Morb Mortal Wkly Rep. 2021 Aug 6;70(31):1059–62. doi:10.15585/mmwr.mm7031e2.
13. Vacharathit V, Aiewsakun P, Manopwisedjaroen S, Srisaowakarn C, Laopanupong T, Ludowyke N, Phuphuakrat A, Setthaudom C, Ekronarongchai S, Srichatrapimuk S, et al. CoronaVac induces lower neutralising activity against variants of concern than natural infection. Lancet Infect Dis. 2021 Aug 26;S1473-3099(21):00568–5.
14. Lumley SF, O'Donnell D, Stoesser NE, Matthews PC, Howarth A, Hatch SB, Marsden BD, Cox S, James T, Warren F, et al. Antibody status and incidence of SARS-CoV-2 infection in health care workers. N Engl J Med. 2021;384:533–40. doi:10.1056/NEJMoa2034545.

CoronaVac

O que a ciência comprova

1.5. CoronaVac protegeu contra 80% das mortes por Covid-19 na Indonésia

Uma pesquisa conduzida em Bali, na Indonésia, atestou que a CoronaVac, vacina de vírus inativado do Butantan e da farmacêutica chinesa Sinovac, foi capaz de conferir uma proteção de mais de 80% contra mortes relacionadas à Covid-19. O trabalho foi publicado na plataforma de preprints MedRxiv e é o primeiro estudo de efetividade do imunizante feito no país – ou seja, com dados de eficácia do mundo real.

Os cientistas analisaram dados de vacinação e de laboratórios de diagnóstico e incluíram no estudo 2.759 pessoas que testaram positivo para Covid-19 entre janeiro e junho de 2021, e 2.759 controles (que não tiveram a doença), todos com idades semelhantes e que foram testados na mesma semana. Dos casos positivos, 40% tinham comorbidades e a maioria não era vacinada (53,8%).

Os resultados apontaram que a CoronaVac teve uma efetividade de 66,7% contra infecções pelo SARS-CoV-2, 71% contra hospitalizações e 87,4% contra mortes. No caso de pessoas acima de 50 anos, a prevenção contra óbitos foi ainda maior, chegando a 90,6%.

A CoronaVac foi a primeira vacina utilizada no programa de imunização contra Covid-19 da Indonésia, em janeiro de 2021. Um estudo clínico de fase 3 conduzido no país já havia mostrado uma eficácia de 65,3% do imunizante contra a doença e soroconversão (produção de anticorpos) em 87% dos vacinados, o que deu suporte para a aprovação de seu uso emergencial.

Estudos conduzidos em outros países, como Chile, Turquia e China, já apresentaram resultados positivos para a CoronaVac. No Brasil, o Projeto S, estudo de efetividade feito pelo Butantan no município de Serrana, em São Paulo, mostrou que a vacina protegeu 80,5% contra casos sintomáticos de Covid-19 e 95% contra hospitalizações e mortes.

Publicado em: 04/02/2022

Effectiveness of the Inactivated COVID-19 Vaccine (CoronaVac) in Adult Population in Bali, Indonesia

Anton Suryatma[1]*, Raras Anasi[1], Miko Hananto[1], Asep Hermawan[1], Ririn Ramadhany[2], Irene Lorinda Indalao[2], Agustiningsih Agustiningsih[2], Ely Hujjatul Fikriyah[1], Teti Tejayanti[1], Rustika Rustika[3], Kristina Lumban Tobing[1], Ketut Suarjaya[4], I Wayan Widia[4], Pandji Wibawa Dhewantara[1]*

[1] Center for Research and Development on Public Health Effort, National Institute of Health Research and Development (NIHRD), Ministry of Health of Indonesia, Jl. Percetakan Negara No. 29, Jakarta 10560, Indonesia

[2] Centre for Research and Development on Biomedical and Basic Health Technology, National Institute of Health Research and Development (NIHRD), Ministry of Health of Indonesia, Jl. Percetakan Negara No. 29, Jakarta 10560, Indonesia

[3] Center for Research and Development on Humanities and Health Management, National Institute of Health Research and Development (NIHRD), Ministry of Health of Indonesia, Jl. Percetakan Negara No. 29, Jakarta 10560, Indonesia

[4] Bali Provincial Health Office, Jl. Melati No. 20, Denpasar 80233, Indonesia

*Authors contributed equally

Correspondence: Anton Suryatma, MD, MPH., Center for Research and Development of Public Health Effort, National Institute of Health Research and Development (NIHRD), Ministry of Health of Indonesia, Jakarta, 10560, Indonesia. Email: drantonsuryatma@gmail.com

40 **Abstract**

41 **Background**: Inactivated SARS-CoV-2 vaccine has been included in the national COVID-19
42 vaccination program in Indonesia since January 2021. The study aims to estimate the
43 effectiveness of CoronaVac vaccine in preventing SARS-CoV-2 infection, hospitalization, and
44 death in adult population aged ≥18 years in Bali, Indonesia.

45 **Methods**: Test-negative, case control study was conducted by linking SARS-CoV-2 laboratory
46 records, COVID-19 vaccination, and health administrative data for the period of January 13 to
47 June 30, 2021, among adults aged ≥18 years in Bali. Case-subjects were defined as individuals
48 who had a positive RT-PCR test for SARS-CoV-2 during the period; they were matched with
49 controls based on age, sex, district of residence, presence of comorbidities and week of testing.
50 Conditional and multivariable logistic regression was performed to estimate adjusted vaccine
51 effectiveness.

52 **Results**:

53 Total 109,050 RT-PCR test results were retrieved during the January 13 to June 30, 2021
54 (Figure 1). Of which, 14,168 subjects were eligible for inclusion in the study. Total 5518
55 matched pairs were analyzed. Adjusted vaccine effectiveness (VE) against laboratory-
56 confirmed SARS-CoV-2 infection was 14.5% (95% confidence interval -11 to 34.2) at 0-13
57 days after the first dose; 66.7% (58.1 to 73.5%) at ≥14 days after the second dose. Adjusted VE
58 in preventing hospitalization and COVID-19-associated death was 71.1% (62.9% to 77.6%)
59 and 87.4% (65.1% to 95.4%) at ≥14 days after receiving the second dose, respectively.

60 **Conclusions**: Two-dose of inactivated CoronaVac vaccine showed high effectiveness against
61 laboratory confirmed COVID-19 infection, hospitalization, and death associated with COVID-
62 19 among adults aged ≥18 years.

63

64 **Keywords**: inactivated vaccine, COVID-19, effectiveness, test-negative, Indonesia

65

Introduction

Indonesia rolled-out the first phase of mass vaccination program on January 13th, 2021, using the inactivated SARS-CoV-2 CoronaVac vaccine (Sinovac Life Sciences, Beijing, China). In the first phase, mass COVID-19 vaccination was targeting healthcare workers and general population including adults and elderly. A phase 1/2 clinical trial in China indicated that CoronaVac was tolerable, with acceptable safety and immunogenicity, and therefore supported the conduct of a phase 3 clinical trial in three countries including Indonesia.[1] The efficacy of CoronaVac varied among the countries, ranging from 50.7% to 84% against SARS-CoV-2 infection.[2-4] In Indonesia, two doses of CoronaVac vaccine had an efficacy of 65.3% against COVID-19 illness after 14 days of injection; this supported the approval of emergency use authorization (EUA) by the Indonesia National Agency of Drug and Food Control (NADFC). [2,5]

Evidence regarding its effectiveness against SARS-CoV-2 infection, hospitalization, and death in the "real world" conditions have been reported in number of countries such as Chile, Turkey, and Brazil.[6,7] However, studies suggest that inactivated COVID-19 vaccine VE varies from region to region. In Indonesia, where inactivated vaccine has been given to the population, study on evaluating inactivated vaccine performance in preventing infection and severe outcomes still limited especially in adults aged ≥ 18 years. The objective of this study was to estimate vaccine effectiveness (VE) of inactivated CoronaVac vaccine against COVID-19 infection, hospitalization, and COVID-19-related death among adult aged ≥18 years in Bali.

Methods

Setting and design

Bali is a one of the popular tropical islands in Indonesia, with a total land area of 5780 km^2 and population of more than 4.3 million.[8] Mass COVID-19 vaccination using inactivated SARS-CoV-2 (CoronaVac) was initiated by the Ministry of Health of Indonesia on January 13, 2021, across Indonesia, including in Bali. As of July 31, 2021, COVID-19 vaccines have been given to 2.8 million (41.9%) of the targeted Balinese population. About 2.2 million people aged ≥18 years had received at least one dose of COVID-19 vaccines (Supplementary Figure S1); 1.2 million (43.1%) had received at least one dose of inactivated CoronaVac vaccine.[9] The observation was conducted during January 13 to June 30, 2021. During the study period, the incidence of COVID-19 was increased during January-February and

followed with a declining trend from March to June 2021. There was a sharp increase in incidence in July due to B.1.617.2 (Delta) variants (Supplementary Figure S2, Figure S3).

We conducted a record-based retrospective, test-negative, matched case control (1:1) study to evaluate the effectiveness of inactivated CoronaVac vaccine in preventing laboratory confirmed SARS-CoV-2 infection, among adults (aged ≥18 years) during January to June 2021. This test negative design was chosen due to several reasons. First, accessibility to individual data who were laboratory-tested for SARS-CoV-2 was feasible through the national surveillance system; second, it was cost-effective and allowed for faster results; and lastly, the design could better control for potential biases, including healthcare seeking behavior and access to COVID-19 testing.[10]

Data sources

We linked individual health electronic databases including SARS-CoV-2 reverse-transcription polymerase chain reaction (RT-PCR) test results from the national laboratory testing database (New All Record), COVID-19 vaccination registry, and hospitalization claims, managed by the Ministry of Health of Indonesia, using unique national citizen identification number. Data with foreign identification number and driving license number were excluded. We also checked the consistency in identification number, name, date of birth and sex between these databases. Confirmatory SARS-CoV-2 PCR test was performed on naso and/or oropharyngeal swab specimens in COVID-19 reference laboratories.[11]

Selection of cases and matched controls

Cases were adults aged ≥18 years identified as those who had at least one COVID-19-like symptom (fever with body temperature documented at ≥38°C, chills, cough, shortness of breath, fatigue or malaise, sore throat, headache, runny nose, congestion, muscle aches, nausea or vomiting, diarrhea, abdominal pain, altered sense of smell or taste) and were tested RT-PCR positive for SARS-CoV-2, between January 13 to June 30, 2021; were not confirmed as positive for SARS COV2 in the last 90 days (about 3 months) before the index date; and those who did not receive any COVID-19 vaccines other than CoronaVac before sample collection. Controls were defined as those who had negative RT-PCR test results during the same period, irrespective for symptoms; and who did not receive any COVID-19 vaccines other than CoronaVac before sample collection. Cases and control were matched based on age, sex, district of residence and week of sample collection/testing.

Vaccination status

Vaccination status of the subjects was determined by referring to linked individual vaccination record and was determined based on their SARS-CoV-2 test date. Fully vaccinated cases and controls were defined as having two doses of CoronaVac ≥14 days before the sample collection date. Adults who received no single dose of CoronaVac vaccine were defined as unvaccinated and all other adults who received one dose ≥14 days before sample collection date were defined as partially vaccinated.

Statistical analysis

Unadjusted and adjusted odds of having received CoronaVac vaccine were estimated using conditional logistic regression. Odds ratios (ORs) and 95% confidence interval (CI) of vaccination in cases and controls was compared. Adjusted ORs were estimated from models including age (by year, as continuous) and presence of comorbidities and prior SARS-CoV-2 infection. There was a considerable proportion of missing data for comorbidities (79%). Multiple imputation was performed with considering for covariates (age, sex, residence) and outcome. Vaccine effectiveness (VE) was calculated as one minus the OR multiplied by 100%. [12] Subgroup analyses were performed to assess VE based on age group (i.e., aged 18-49 years vs. 50 years and older). Additional analysis was performed to estimate VE against secondary outcomes (hospitalization and death due to COVID-19) using multivariable logistic regression, accounting for covariates such as age, sex, district of residence, imputed presence of comorbidities and week of sample collection. All statistical analyses were conducted using STATA software version 15.0 (Stata Cooperation, College Station, Texas, USA).

Results

Study population

Total 109,050 RT-PCR test results were retrieved during the January 13 to June 30, 2021 (Figure 1). Of which, 14,168 subjects were eligible for inclusion in the study. Of these, 2886 (20.4%) were having positive RT-PCR test for SARS-CoV-2 and 11,282 (79.6%) were tested negative for SARS-CoV-2 infection. From this, total 5518 (39%) subjects were defined as case-control pairs after matching.

Characteristics of the study population and matched case-control were presented in Table 1. The median age of the overall study population (n=14,168) was 50 years (interquartile range, 31-65 years); 50.8% were aged ≥50 years. Thirty percent (n=4259) resided in Denpasar city.

Thirty four percent (n=4863) had received full vaccination; 42.5% (n=6016) had not received vaccine. Those who receiving positive RT-PCR test results were appeared much older, males, had at least one comorbidity and unvaccinated.

Among the case subjects, about 40% of subjects having comorbidities. Based on vaccination status, 453 (16.4%) had received two doses of CoronaVac vaccine. Nineteen percent (n=535) case subjects had received the first dose of CoronaVac vaccine and about two-third (n=1771) were unvaccinated. While among the control subjects, half of the subjects (n=1485/2759) were unvaccinated. Twenty four percent had received two doses of CoronaVac vaccine.

Vaccine effectiveness

After adjusting for covariates, compared to unvaccinated adults, two-dose of CoronaVac improved protection against SARS-CoV-2 infection (VE: 66.7%; 95%CI: 58.1-73.5%) at ≥14 days after the second dose. Partial vaccination with CoronaVac was not significantly associated with a reduced risk of laboratory-confirmed SARS-CoV-2 infection (VE: 14.5%; 95%CI: -11 to 34.2) at 0-13 days after the first dose. CoronaVac VE against COVID-19 infection was reduced with increasing age; 66.6% to 84.2% in adults aged 18-49 years vs. 57.7% to 78.7% in adults aged ≥50 years (Supplementary Table S1).

The adjusted VE in preventing COVID-19-related hospitalization and death was 71.1% (95%CI: 62.9%-77.6%) and 87.4% (95%CI: 65.1%-95.4%), respectively, at 14 days after the second dose (Table 3). Partial vaccination was less protective against COVID-19-related hospitalization and death among people aged 18 and older (Supplementary Table S2). Vaccine effectiveness against hospitalization was higher 79.4% (95%CI: 70-85.9%) in group of people aged ≥50 years relative to people aged 18-49 years (65.8%; 95%CI:49.1-77%). Death due to COVID-19 was not significantly associated with full vaccination in people aged 18-49 years (VE:65.7%, 95%CI: -99.5% to 94.1%). This result could be happened due to an effect of small sample size and small number of events (mortality). Among older population, vaccine effectiveness was 90.6% (95%CI:61.5-97.7%) in preventing death due to COVID-19.

To address potential issues related to the small sample size and rare event or outcome, we conducted an additional analysis using study population (n=14,168) (Supplementary Table S3, Table S4). Overall, the vaccine effectiveness was 65.4% (95%CI: 59.5-70.4%) in preventing COVID-19 infection; 78.1% (95%CI: 73.6-81.9%) in preventing hospitalization; and 93.9% (95%CI: 85-97.6%) in preventing COVID-19-related death in adults aged 18 years or older at 14-days after second dose. The effectiveness against both hospitalization and death due to

COVID-19 was higher in that population aged 50 years or older (71.9% and 83.1%, respectively) compared to group of people aged 18-49 years (88.7% and 94.1%, respectively) (Supplementary Table S4).

Discussion

High proportion of inactivated vaccine of CoronaVac have been used in national COVID-19 vaccination program in Indonesia. So far, this is the first health record-based observational study on assessing 'real-world' COVID-19 vaccine performance against laboratory-confirmed SARS-CoV-2 symptomatic infection, hospitalization, and death in Indonesian population. The study showed that complete regimen of CoronaVac vaccine appeared to be protective against COVID-19 infection about 66.7% (58.1-73.5%) among Balinese adults aged ≥18 years, which is consistent with the results of phase III trial.[5] Yet, the effectiveness was reduced by age. Furthermore, vaccination with CoronaVac vaccine was highly effective in preventing COVID-19-associated hospitalizations and death. This demonstrates that CoronaVac vaccination could reduce the risk of development severe COVID-19, especially in older population.

Our 'real world' estimated VE against symptomatic COVID-19 infection was consistent with that randomized controlled trials of vaccine efficacy in Indonesia (65.3%; 95%CI: 20%-85.1%).[2] Yet, it was lower than that of vaccine efficacy reported in Turkey (83.5%; 95% CI: 65.4%-92.1%)[3] and higher than that efficacy reported from Brazil (51%; 95%CI: 36%-62%) at 14 days after the second vaccination.[13] Chilean cohort VE study involving general population aged ≥16 years old also demonstrated the VE was 65.9% (95%CI: 65.2%-66.6%) against COVID-19 infection. While a TND case control study in Brazil demonstrated that two dose regimens of CoronaVac was effective in reducing risk of symptomatic COVID-19 (46.8%; 95%CI: 38.7% to 53.8%) at ≥14 days after the second dose among older population aged ≥70 years.[7]

Our study showed that inactivated CoronaVac vaccine was also effective in preventing hospitalization and COVID-19-associated death. We estimated that CoronaVac vaccine was 71.1% for the prevention of hospitalization and 87.4% for the prevention of COVID-19-related death. None has reported CoronaVac VE estimates against these two outcomes in Indonesia. Our VE estimates are dissimilar to estimates that have been reported elsewhere. A cohort study in Chile among adults aged ≥16 years showed that CoronaVac vaccine was 87.5% (95% CI, 86.7 to 88.2) effective against hospitalization and 86.3% (95% CI, 84.5 to 87.9) effective against COVID-19–related death.[6,14] While a TND study in Brazil among elderly aged 70 years or older, CoronaVac was effective against COVID-19-related hospitalization (55.5%; 95%CI:

46.5% to 62.9%) and death (61.2%, 95%CI: 48.9% to 70.5%) at ≥14 days after the second dose, respectively.[7] This discrepancy might be due to several factors including variation in study population and design (e.g., targeted age group, sample size) and difference in risk of transmission or epidemiological setting (e.g., a TND study in Brazil was conducted when P.1 or Gamma variant was predominant).

The strengths of this study are that: i) we used comprehensive health administrative database including individual laboratory test results, vaccination data and hospitalizations which include information on date of sample collection, type of vaccine, date of vaccination, date of hospital admission and underlying conditions; (ii) the subjects were chosen based on a standard laboratory confirmation for SARS-CoV-2 results. Thus, biases due to misclassification from infection or vaccination status were implausible. These findings are important to fill the gap in our knowledge regarding the performance of inactivated CoronaVac vaccine in the real-world. However, our study has limitations. First, while we attempted to make a robust approach by adjusting for number of potential confounding factors, including age, sex, geography, week of testing (sample collection) and presence of comorbidities, there might be still unmeasured confounding factors that biased the VE estimates (e.g., behavioral risk factors, type of variants). Second, this study was retrieved data from January to June 2021, which was just before the peak of Delta variants (B.1.617.2) outbreaks. Hence, the VE estimates presented here is likely reflected vaccine performance before Delta and Omicron becoming predominant variant in the community. VE of CoronaVac against Delta and Omicron-related infection and severity might be different. Studies elsewhere have reported the effects of Delta variants on the COVID-19 vaccines performance.[15,16] Further research is required to evaluate the effectiveness of CoronaVac against the circulating VOCs.

Conclusions

The two-dose regimens of inactivated CoronaVac vaccine was effective in preventing COVID-19 infection, hospitalizations for COVID-19 and COVID-19-related death in adults aged 18 years or older. Full vaccination was significantly reduced the risk of severe outcomes especially among 50 years or older.

Author contributions

Study conceptualization: AS, PWD, RR (Ririn Ramadhany), MH. Methodology: AS, RA, RR (Ririn Ramadhany), MH, AH, EHF, PWD. Formal analysis: AS, RA, PWD, MH. Project

administration: AS, RA, RR (Rustika Rustika), TT, AA, KS, IWW. Data collection: AS, RA, EHF, AA, KS, ILI, IWW, PWD. Validation: AS, RA, PWD, AS, RR (Ririn Ramadhany), ILI, AA, RR (Rustika Rustika), KK, TT. Funding: AS, TT, MH. Writing (original draft): AS, PWD. Writing (review and editing): all authors.

Ethic statement

The protocol of the study was approved by the Health Research Ethics Committee, National Institute of Health Research and Development, Ministry of Health, Indonesia (LB. 02.01/2/KE.533/2021). The study was considered exempt from informed consent. No human health risks were identified.

Conflict of interest

The authors declare that they have no competing interests. Anton Suryatma, Raras Anasi, Miko Hananto, Asep Hermawan, Ririn Ramadhany, Irene Lorinda Indalao, Agustiningsih Agustiningsih, Ely Hujjatul Fikriyah, Teti Tejayanti, Rustika Rustika, Kristina Lumban Tobing and Pandji Wibawa Dhewantara are full-time researcher at the National Institute of Health Research and Development, Ministry of Health of Indonesia. Ketut Suarjaya and I Wayan Widia are full-time employees of Provincial Health Office in Bali. None of us received shares or any kind monetary compensation linked to the distribution of CoronaVac in Indonesia or have any share or financial interests in Sinovac Life Sciences or parent companies.

Data availability statement

The datasets used and/or analyzed during the current study are protected by the Ministry of Health of Indonesia and are unsuitable for public sharing. Interested parties can apply for the data by contacting the data center of the Ministry of Health of Indonesia. All data generated or analyzed during this study are included in this published article (and its Supplementary information files). Aggregate data on vaccination and COVID-19 incidence are publicly available at https://vaksin.kemkes.go.id/#/vaccines

Funding

This study was funded by the National Institute of Health Research and Development, Ministry of Health, Indonesia. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The views expressed in this publication are those of the authors and not necessarily those of the Ministry of Health of Indonesia.

Acknowledgement

We would like to thank Dr. Anas Ma'ruf and team at the Center of Health Data and Information of Ministry of Health Indonesia, and Mr. Made Darmawan and Mr. Akbar Antony Siregar at the National COVID-19 Control and Economic Recovery Committee (KPC-PEN) for their assistance and support in collating the data. We would also like to thank the Director of Provincial Health Office of Bali and team for their support in the implementation of this study and permission to access COVID-19 surveillance data.

References

1. Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *The Lancet Infectious diseases.* 2021;21(2):181-192.
2. Indonesia National Agency of Drug and Food Control. Emergency Use Authorization - CoronaVac. In. Jakarta: Indonesia National Agency of Drug and Food Control 2021.
3. Tanriover MD, Doğanay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *Lancet.* 2021;398(10296):213-222.
4. Palacios R, Patiño EG, de Oliveira Piorelli R, et al. Double-Blind, Randomized, Placebo-Controlled Phase III Clinical Trial to Evaluate the Efficacy and Safety of treating Healthcare Professionals with the Adsorbed COVID-19 (Inactivated) Vaccine Manufactured by Sinovac - PROFISCOV: A structured summary of a study protocol for a randomised controlled trial. *Trials.* 2020;21(1):853-853.
5. Fadlyana E, Rusmil K, Tarigan R, et al. A phase III, observer-blind, randomized, placebo-controlled study of the efficacy, safety, and immunogenicity of SARS-CoV-2 inactivated vaccine in healthy adults aged 18-59 years: An interim analysis in Indonesia. *Vaccine.* 2021;39(44):6520-6528.
6. Ministerio de Salud Gobierno de Chile. *Effectiveness of the inactivated CoronaVac vaccine against SARS-CoV-2 in Chile - Preliminary report.* Chile: Misterio de Salud;2021.
7. Ranzani OT, Hitchings MDT, Dorion M, et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study. *BMJ (Clinical research ed).* 2021;374:n2015.
8. Provincial Bureau of Statistics of Bali. *Bali in Figure 2021*. Denpasar: Provincial Bureau of Statistics of Bali; 2021.
9. Ministry of Health of Indonesia. COVID-19 Vaccine Dashboard. 2021; https://vaksin.kemkes.go.id/#/vaccines. Accessed 3 August 2021, 2021.
10. Patel MK, Bergeri I, Bresee JS, et al. Evaluation of post-introduction COVID-19 vaccine effectiveness: Summary of interim guidance of the World Health Organization. *Vaccine.* 2021;39(30):4013-4024.
11. Ministry of Health of Indonesia. *Guidelines on COVID-19 Prevention and Control, 5th revision* Jakarta: Ministry of Health of Indonesia; 2020.

12. Orenstein WA, Bernier RH, Dondero TJ, et al. Field evaluation of vaccine efficacy. *Bull World Health Organ.* 1985;63(6):1055-1068.
13. World Health Organization. *Interim recommendations for use of the inactivated COVID-19 vaccine, CoronaVac, developed by Sinovac (Interim Guidance 24 May 2021).* Geneva: World Health Organization; 2021.
14. Jara A, Undurraga EA, González C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *New England Journal of Medicine.* 2021;385(10):875-884.
15. Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. *N Engl J Med.* 2021;385(7):585-594.
16. Li X-N, Huang Y, Wang W, et al. Effectiveness of inactivated SARS-CoV-2 vaccines against the Delta variant infection in Guangzhou: a test-negative case-control real-world study. *Emerg Microbes Infect.* 2021;10(1):1751-1759.

12. Orenstein WA, Bernier RH, Dondero TJ, et al. Field evaluation of vaccine efficacy. *Bull World Health Organ.* 1985;63(6):1055-1068.
13. World Health Organization. *Interim recommendations for use of the inactivated COVID-19 vaccine, CoronaVac, developed by Sinovac (Interim Guidance 24 May 2021).* Geneva: World Health Organization; 2021.
14. Jara A, Undurraga EA, González C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *New England Journal of Medicine.* 2021;385(10):875-884.
15. Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. *N Engl J Med.* 2021;385(7):585-594.
16. Li X-N, Huang Y, Wang W, et al. Effectiveness of inactivated SARS-CoV-2 vaccines against the Delta variant infection in Guangzhou: a test-negative case-control real-world study. *Emerg Microbes Infect.* 2021;10(1):1751-1759.

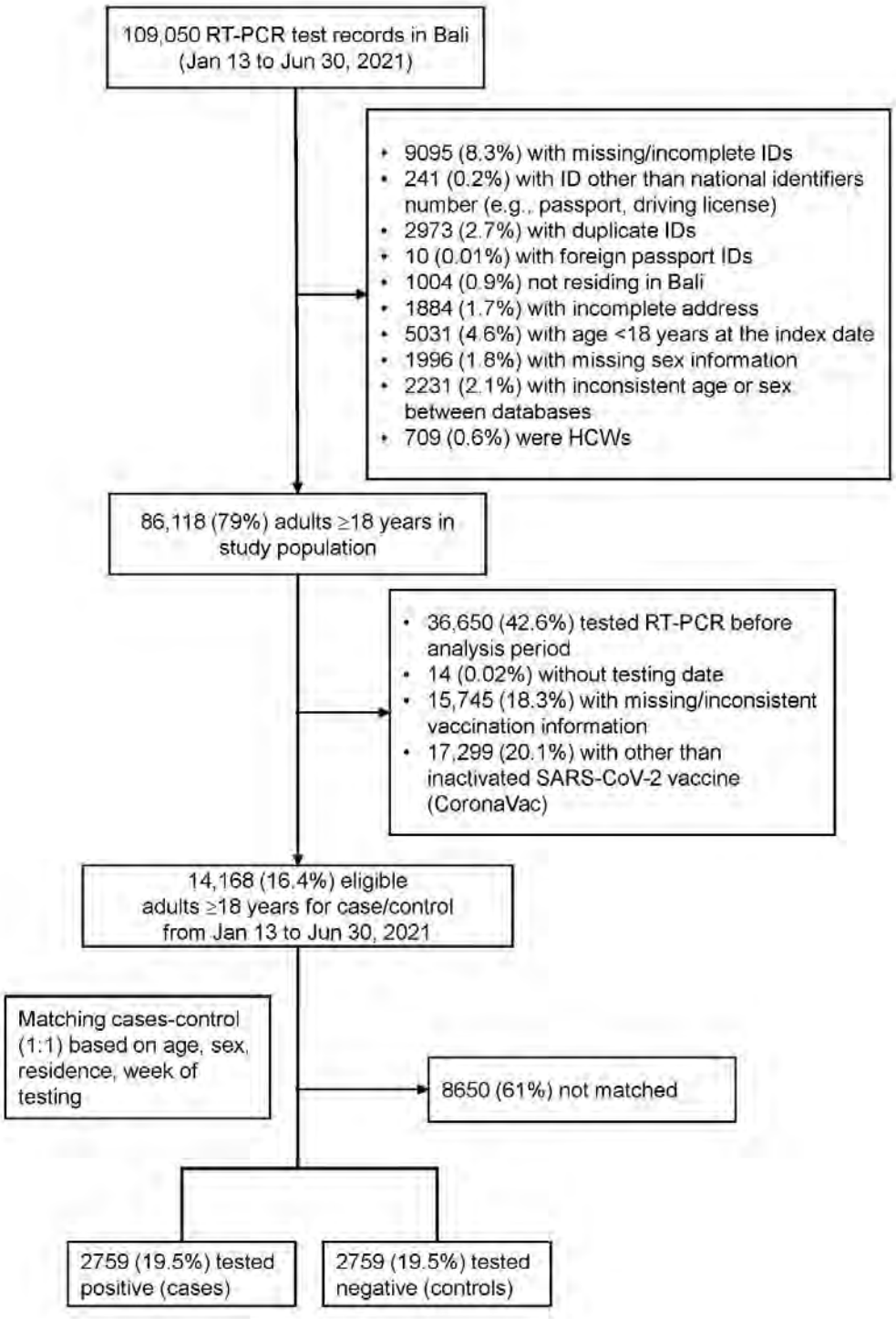


Figure 1. Flowchart for case and control selection

395 **Table 1.** Characteristics of the adults aged ≥18 years eligible in the study and case-control
396 pairs included in the analysis
397

Characteristics	Total	Eligible study population		Matched pairs	
		Positive SARS-CoV-2 (n=2886)	Negative SARS-CoV-2 (n=11,282)	Cases (n=2759)	Controls (n=2759)
Age, years, median (IQR)	50 (31-65)	61 (45-69)	46 (30-64)	61 (45-69)	60 (41-70)
Age group, n (%)					
18-49 y	6969 (49.2)	853 (29.6)	6116 (54.2)	847 (30.7)	847 (30.7)
≥50 y	7199 (50.8)	2033 (70.4)	5166 (45.8)	1912 (69.3)	1912 (69.3)
Sex					
Male	7676 (54.2)	1730 (59.9)	5946 (52.7)	1648 (59.7)	1648 (59.7)
Female	6492 (45.8)	1156 (40.1)	5336 (47.3)	1111 (40.3)	1111 (40.3)
District of residence, n (%)					
Jembrana	564 (4)	87 (3)	477 (4.2)	75 (2.7)	75 (2.7)
Tabanan	1500 (10.6)	233 (8.1)	1267 (11.2)	233 (8.5)	233 (8.5)
Badung	2031 (14.3)	474 (16.4)	1557 (13.8)	465 (16.7)	456 (16.7)
Gianyar	1573 (11.1)	408 (14.1)	1165 (10.3)	363 (13.2)	363 (13.2)
Klungkung	554 (3.9)	79 (2.7)	475 (4.2)	73 (2.7)	73 (2.7)
Bangli	803 (5.7)	222 (7.7)	581 (5.2)	202 (7.3)	202 (7.3)
Karangasem	916 (6.5)	144 (5)	772 (6.8)	138 (5)	138 (5)
Buleleng	1968 (13.9)	353 (12.2)	1615 (14.3)	343 (12.4)	343 (12.4)
Denpasar	4259 (30.1)	886 (30.7)	3373 (29.9)	867 (31.4)	867 (31.4)
Reported No. of comorbidities, n (%)					
No	12,158 (85.8)	1698 (58.8)	10,460 (92.7)	1636 (59.3)	2473 (89.6)
Yes	2010 (14.2)	1188 (41.2)	822 (7.3)	1123 (40.7)	286 (10.4)
Vaccination status, n (%)					
Unvaccinated	6016 (42.5)	1867 (64.7)	4149 (36.8)	1771 (64.2)	1469 (53.3)
1st dose (0-13 days)	1001 (7.1)	222 (7.7)	779 (6.9)	216 (7.8)	193 (7)
1st dose (≥14 days)	1408 (9.9)	340 (11.8)	1068 (9.5)	319 (11.6)	272 (9.9)
2nd dose (0-13 days)	880 (6.2)	77 (2.7)	803 (7.1)	74 (2.7)	158 (5.7)
2nd dose (≥14 days)	4863 (34.3)	380 (13.2)	4483 (39.7)	379 (13.7)	667 (24.2)

398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416

Table 2. CoronaVac vaccine effectiveness against laboratory-confirmed SARS-CoV-2 infection in adults aged ≥18 years in Bali, Indonesia

Vaccination status	Cases	Controls	Vaccine effectiveness (95% CI)	
			Unadjusted	Adjusted*
Unvaccinated	1771 (64.2)	1469 (53.3)	Ref	Ref
0-13 days after 1st dose	216 (7.8)	193 (7)	22.9 (2.4- 39)	14.5 (-11 – 34.2)
≥14 days after 1st dose	319 (11.6)	272 (9.9)	24.1 (7.4-37.8)	10.5 (-12 – 28.6)
0-13 days after 2nd dose	74 (2.7)	158 (5.7)	67 (55.3-75.6)	63.1 (48.5-73.6)
≥14 days after 2nd dose	379 (13.7)	667 (24.2)	73.4 (78.4-67.2)	66.7 (58.1-73.5)

*Included covariates: age (as continuous), presence of comorbidities and prior SARS-CoV-2 infection

Table 3. CoronaVac vaccine effectiveness in preventing hospitalization and COVID-19-related death in adults aged ≥18 years in Bali, Indonesia

Vaccination status	Hospitalization		VE* % (95%CI)	Death		VE* % (95%CI)
	Yes (n=2490)	No (n=3028)		Yes (n=482)	No (n=5036)	
Unvaccinated	1932 (77.6)	1308 (43.2)	Ref	450 (93.4)	2790 (55.4)	Ref
0-13 days after 1st dose	124 (5)	285 (9.4)	28.3 (5.3-45.7)	10 (2.1)	399 (7.9)	53.9 (10-76.3)
≥14 days after 1st dose	218 (8.8)	373 (12.3)	34.1 (16.4-48.1)	15 (3.1)	576 (11.4)	58.6 (28.3-76.1)
0-13 days after 2nd dose	50 (2)	182 (6)	61.2 (42.6-73.7)	3 (0.6)	229 (4.6)	76 (22.9-92.5)
≥14 days after 2nd dose	166 (6.7)	880 (29)	71.1 (62.9-77.6)	4 (0.8)	1042 (20.7)	87.4 (65.1-95.4)

Abbreviations: VE, vaccine effectiveness; CI, confidence interval

*Adjusted by age (as continuous), sex, presence of comorbidities and prior SARS-CoV-2 infection, week of testing, district of residence

Supplementary file

Table S1. CoronaVac vaccine effectiveness against laboratory-confirmed SARS-CoV-2 infection in adults (aged ≥18 years), by age group, in Bali, Indonesia

Vaccination status	Cases (n=2759)	Controls (n=2759)	VE % (95%CI)	
			Unadjusted	Adjusted*
Age group: 18-49 years (n=1694)				
Unvaccinated	247 (29.2)	95 (11.2)	Ref	Ref
0-13 days after 1st dose	133 (15.7)	122 (14.4)	57.2 (37.6-70.6)	48.3 (22.6-65.5)
≥14 days after 1st dose	144 (17)	131 (15.5)	65.7 (49.4-76.8)	55.1 (32-70.4)
0-13 days after 2nd dose	39 (4.6)	79 (9.3)	80.3 (68-87.9)	78.2 (63.6-87)
≥14 days after 2nd dose	284 (33.5)	420 (49.6)	82.2 (74.5-87.5)	77 (66.6-84.2)
Age group: ≥50 years (n=3824)				
Unvaccinated	1524 (79.7)	1374 (71.9)	Ref	Ref
0-13 days after 1st dose	83 (4.3)	71 (3.7)	-5 (-46.6–24.8)	-10.6 (-64.3–25.5)
≥14 days after 1st dose	175 (9.2)	141 (7.4)	-2 (-30 – 20)	-13.2 (-50.6 – 14.9)
0-13 days after 2nd dose	35 (1.8)	79 (4.1)	61.7 (42.3-74.6)	58.1 (32.4-74)
≥14 days after 2nd dose	95 (4.9)	247 (12.9)	74.3 (65.2-80.9)	69.9 (57.7-78.7)

*Included covariates: age (as continuous), presence of comorbidities and prior SARS-CoV-2 infection

Table S2. CoronaVac vaccine effectiveness in preventing hospitalization and death due to COVID-19 in adults (aged ≥18 years), by age group, in Bali, Indonesia (n=5518)

Vaccination status	Hospitalization		VE* % (95%CI)	Death		VE* % (95%CI)
	Yes (n=2490)	No (n=3028)		Yes (n=482)	No (n=5036)	
Age group: 18-49 years (n=1694)						
Unvaccinated	123	219	Ref	11	331	Ref
0-13 days after 1st dose	53	202	34.5 (-1-57.5)	0	255	NA
≥14 days after 1st dose	65	210	32.9 (-1.9-55.8)	1	274	78.7 (-84.6-97.6)
0-13 days after 2nd dose	16	102	64.9 (32.7-81.7)	1	117	51.3 (-330-94.5)
≥14 days after 2nd dose	99	605	65.8 (49.1-77)	2	702	65.7 (-99.5-94.1)
Age group: ≥50 years (n=3824)						
Unvaccinated	1809	1089	Ref	439	2459	Ref
0-13 days after 1st dose	71	83	14.1 (-28.1-42.4)	10	144	35.7 (-26.1-67.3)
≥14 days after 1st dose	153	163	34.7 (11.2-51.9)	14	302	57.2 (24.7-75.7)
0-13 days after 2nd dose	34	80	55.1 (26.5-72.6)	2	112	78.9 (12.7-94.9)
≥14 days after 2nd dose	67	275	79.4 (70-85.9)	2	340	90.6 (61.5-97.7)

Table S3. Assessment of CoronaVac vaccine effectiveness against laboratory-confirmed SARS-CoV-2 infection, hospitalization and death in adults aged ≥18 years in Bali, Indonesia, based on study population (n = 14,168)

Vaccination status	COVID-19 (n/N)	VE* % (95%CI)	Hospitalization (n/N)	VE* % (95%CI)	COVID-19-related death (n/N)	VE* % (95%CI)
Unvaccinated	1867/6016	Ref	2841/6016	Ref	574/6016	Ref
0-13 days after 1st dose	222/1001	6.7 (-12.1-22.3)	158/1001	27.9 (8.7-43.1)	10/1001	64.5 (31.6-81.5)
≥14 days after 1st dose	340/1408	-6.2 (-24.4-9.3)	336/1408	32 (17.4-44)	19/1408	62.9 (39.9-77.1)
0-13 days after 2nd dose	77/880	65 (54.7-72.9)	75/880	65.6 (53.3-74.7)	3/880	84.2 (50-95)
≥14 days after 2nd dose	380/4863	65.4 (59.5-70.4)	308/4863	78.1 (73.6-81.9)	5/4863	93.9 (85-97.6)

*Based on adjusted multivariable logistic regression, accounting for age (continuous), sex, district of residence, week of testing, presence of comorbidities, and prior infection.

Table S4. CoronaVac vaccine effectiveness in preventing hospitalization and death due to COVID-19 in adults (aged ≥18 years), by age group, in Bali, Indonesia based on the study population (n = 14,168)

Vaccination status	Hospitalization		VE* % (95%CI)	Death		VE* % (95%CI)
	Yes (n=3718)	No (n=10,450)		Yes (n=482)	No (n=5036)	
Age group: 18-49 years (n=1694)						
Unvaccinated	187	689	Ref	14	862	Ref
0-13 days after 1st dose	73	691	36 (8.2-55.4)	0	764	NA
≥14 days after 1st dose	109	749	35.8 (9.3-54.5)	1	857	88 (1.6-98.5)
0-13 days after 2nd dose	26	659	75.3 (59.4-85)	1	684	73.6 (-117.7-96.8)
≥14 days after 2nd dose	178	3608	71.9 (61.6-79.5)	2	3784	88.7 (36.5-98)
Age group: ≥50 years (n=3824)						
Unvaccinated	2654	2486	Ref	560	4580	Ref
0-13 days after 1st dose	85	152	12.9 (-22.6-38.1)	10	227	44.4 (-7.7-71.2)
≥14 days after 1st dose	227	323	26.6 (6.4-42.5)	18	532	57.8 (30.9-74.3)
0-13 days after 2nd dose	49	146	48 (21.8-65.4)	2	193	83.9 (34.1-96.1)
≥14 days after 2nd dose	130	947	83.1 (77.9-87.1)	3	1077	94.1 (81.4-98.1)

*Based on adjusted multivariable logistic regression, accounting for age (continuous), sex, district of residence, week of testing, presence of comorbidities, and prior infection.

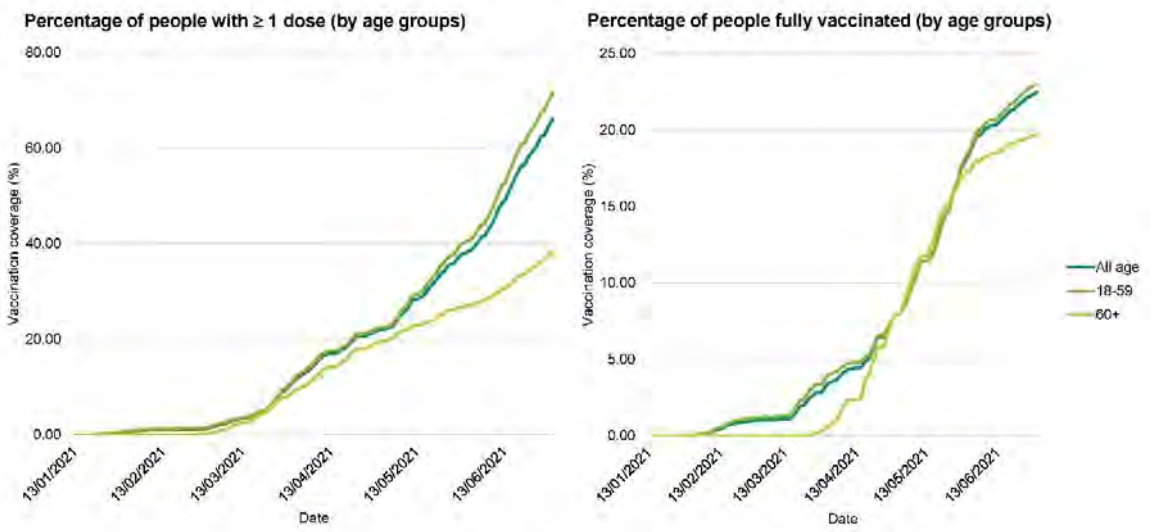


Figure S1. COVID-19 vaccination coverage in adults ≥18 years (any type of vaccines) during January 13 to June 30, 2021, in Bali, Indonesia (Data source: Ministry of Health of Indonesia).

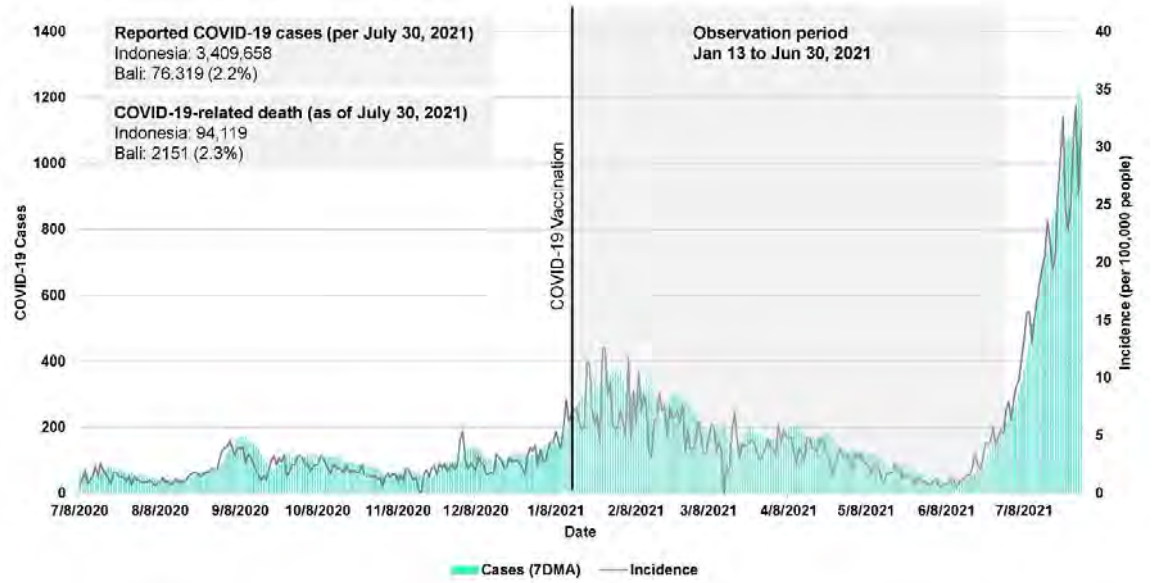


Figure S2. Trends in COVID-19 incidence in Bali over the period of study (Data source: Ministry of Health of Indonesia).

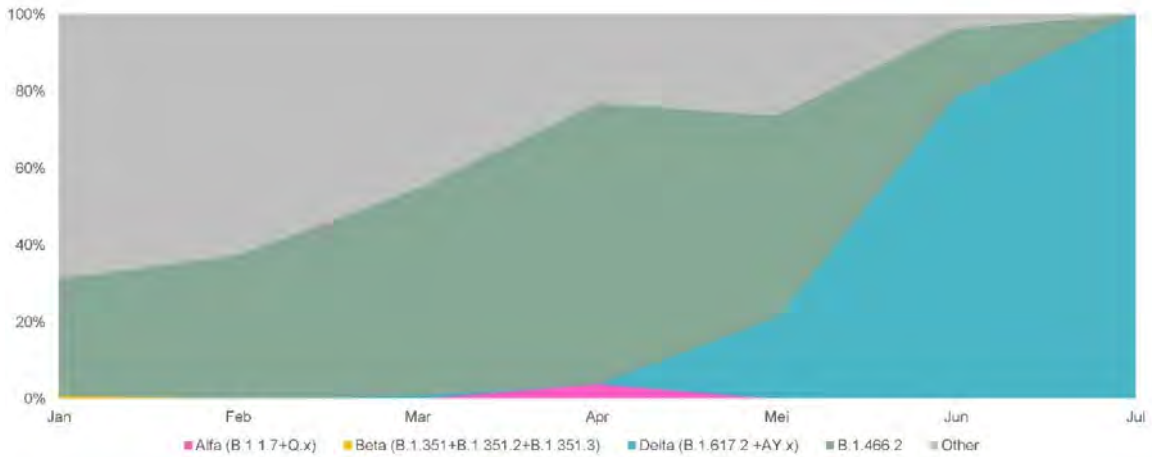


Figure S3. Distribution of SARS-CoV-2 variants of concern (VOCs) in samples examined during January to July 2021 in Bali, Indonesia. Variant proportions are estimated against total sequences of collected samples of each month. Total samples are varied for each month. Data are subject to change over time and will be updated as more data become available. Variant proportions may not represent national estimates. Analysis is based on data submitted by Indonesia Genomic Laboratory Surveillance Network as of 22 September 2021 (Data source: Ministry of Health of Indonesia).

CoronaVac

O que a ciência comprova

1.6. Estudo mostra 99,9% de efetividade da CoronaVac na Amazônia colombiana

Uma pesquisa publicada na revista Tropical Diseases, Travel Medicine and Vaccines apontou que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, foi 99,9% efetiva para evitar casos graves da Covid-19 em uma população da região amazônica da Colômbia, além de oferecer proteção de 94,3% contra casos leves da doença.

O estudo descritivo observacional foi conduzido entre fevereiro e agosto de 2021 no município de Mitú, em Vaupés, com 7.849 indivíduos acima de 18 anos imunizados com a CoronaVac – o equivalente a 99% da população. O local foi priorizado na campanha de vacinação devido à proximidade com o estado brasileiro do Amazonas, onde surgiu a variante gama (P.1) do SARS-CoV-2.

As análises mostraram que, após a imunização, 5,7% dos vacinados tiveram Covid-19 e apenas 0,1% precisaram de hospitalização.

Nos infectados com menos de 60 anos (406), 405 desenvolveram sintomas leves e apenas um teve sintomas moderados. Já nos indivíduos idosos (41), 40 apresentaram infecção leve e um teve a doença grave.

Queda de casos e de mortalidade

Em maio de 2021, houve um novo pico de 200 casos de Covid-19 em Mitú. "Essa onda foi muito menor do que a de agosto de 2020, quando foram reportados 327 casos", apontam os pesquisadores no artigo. A taxa de mortalidade também foi reduzida de 2,2% para 0,22% na comparação entre os dois períodos.

Além disso, quando foi atingido o pico de indivíduos imunizados, houve redução de 72% nos casos de Covid-19 no município. Os cientistas destacam que os casos na população vacinada de Mitú podem ser atribuídos à alta circulação da variante gama na época. No entanto, o estudo mostra que a CoronaVac foi capaz de controlar a gravidade dos casos e a mortalidade relacionadas a essa cepa.

O imunizante do Butantan e da Sinovac representa 40% das vacinas contra Covid-19 utilizadas na Colômbia e já teve mais de 1,8 bilhão de doses aplicadas em todo o mundo. No Brasil, foram 100 milhões de doses.

"Essa plataforma vacinal consiste no vírus SARS-CoV-2 inativado e já teve segurança, efetividade e imunogenicidade comprovadas. A estratégia também foi usada com sucesso em Serrana, no Brasil", informa o artigo, referindo-se aos resultados do Projeto S, estudo clínico de efetividade conduzido pelo Butantan no município do interior de São Paulo.

Publicado em: 15/01/2022

Artigo
Tropical Diseases, Travel Medicine and Vaccines

Serrano-Coll *et al. Tropical Diseases, Travel Medicine and Vaccines* (2022) 8:2
https://doi.org/10.1186/s40794-021-00159-x

SHORT REPORT Open Access

Effectiveness of the CoronaVac® vaccine in a region of the Colombian Amazon, was herd immunity achieved?

Héctor Serrano-Coll[1,2], Hollman Miller[3], Camilo Guzmán[1], Ricardo Rivero[1], Bertha Gastelbondo[1], Jorge Miranda[1], Ketty Galeano[1], Jhon Montaña-Restrepo[3] and Salim Mattar[1*]

Abstract

Introduction: Currently, more than 4.5 billion doses of SARS-CoV-2 vaccines have been applied worldwide. However, some developing countries are still a long way from achieving herd immunity through vaccination. In some territories, such as the Colombian Amazon, mass immunization strategies have been implemented with the CoronaVac® vaccine. Due to its proximity to Brazil, where one of the variants of interest of SARS-CoV-2 circulates.

Objective: To determine the effectiveness of the CoronaVac® vaccine in a population of the Colombian Amazon.

Methods: Between February 24, 2021, and August 10, 2021, a descriptive observational study was carried out in which a population of individuals over 18 years of age immunized with two doses of the CoronaVac® vaccine was evaluated. The study site was in the municipality of Mitú, Vaupés, in southeastern Colombia, a region located in the Amazon bordering Brazil. Results. 99% of the urban population of the Mitú municipality were vaccinated with CoronaVac®. To date, 5.7% of vaccinated individuals have become ill, and only 0.1% of these require hospitalization. One death was attributable to COVID-19 has been reported among vaccinated individuals, and the vaccine has shown 94.3% effectiveness against mild disease and 99.9% against severe infection.

Conclusions: The herd immunity achieved through mass vaccination in this population has made it possible to reduce the rate of complicated cases and mortality from COVID-19 in this region of the Colombian Amazon.

Highlights:

- CoronaVac® has shown 94.3% effectiveness against mild disease and 99.9% against severe infection in this indigenous population.
- CoronaVac® reduces the mortality rate from 2.2% in 2020 to 0.22% in 2021.
- The herd immunity was achieved through mass vaccination in this region of the Colombian Amazon.

Keywords: COVID-19 vaccines, Prevention, Post-exposure, Prophylaxis, Public health, Mass vaccination

* Correspondence: smattar@correo.unicordoba.edu.co
[1]Universidad de Córdoba, Instituto de Investigaciones Biológicas del Trópico, Montería, Colombia
Full list of author information is available at the end of the article

Introduction

Currently, around 168 million cases and more than three million deaths from Coronavirus disease 2019 (COVID-19) have been reported, and more than 4.5 billion doses of vaccines against SARS-CoV-2 have been applied worldwide (August 11, 2021) [1]. However, only 26.6% of its population has been fully immunized in developing countries such as Colombia, so herd immunity is still far from being achieved (August 11, 2021) [2]. The proximity to countries such as Brazil, where the appearance of the P.1 variant has endangered the health system of this country [3], Colombian Amazon was prioritized with the vaccination's program.

Due to storage and transportation facilities, the CoronaVac® vaccine (Sinovac, China) was chosen for mass immunization in tropical regions of Colombia, such as the Amazon. This vaccine platform consists of a chemically inactivated SARS-CoV-2 virus and has proven to be safe, effective, and immunogenic against this new virus, and around 100 million doses of this vaccine have been applied worldwide [4]. Furthermore, this strategy of vaccination using CoronaVac® was used successfully in a small population in Serrana, Brazil [5]. Therefore, this vaccination strategy could be relevant to mitigate the spread of SARS-CoV-2 in small and remote communities in Latin America.

On the other hand, as of august 10, 2021, Colombia has received 13,299,364 vaccines against COVID-19; 7,872,675 (40.1%) from Sinovac, 7,872,440 (40.24%) from Pfizer-Biotech, 2,085,073 (10.66%) from AstraZeneca, 1,171,453 (5,99%) from Janssen, and 608.142 (3.11) From Moderna, and it is essential to note that of the total number of vaccines applied in this country to date, 40% corresponds to CoronaVac® [6].

This work aimed was to determine the effectiveness of the CoronaVac® vaccine in a population of the Colombian Amazon.

Methods

A descriptive observational study was carried out in which a population of individuals older than 18 years immunized with two doses of the CoronaVac® vaccine (Sinovac, China) was evaluated. The study period was between February 24, 2021, to August 10, 2021. The work was developed in the municipality of Mitú, Vaupés, Colombia, a region located in the southeast of Colombia (Amazonas) bordering Brazil (Fig. 1). Mitú is the capital of Vaupés and has 7856 inhabitants, immunized with two doses with an interval of 20 days with the CoronaVac® vaccine that uses SARS-CoV-2 chemically inactivated with beta-Propiolactone [7, 8]. Sociodemographic and clinical characteristics and vaccination data of patients were obtained from secondary sources as a raw database supplied by the Mitu municipality's health secretary. The primary outcome of this study was to evaluate the effectiveness of CoronaVac® in reducing mortality and severe illness due to SARS-CoV-2 in individuals with a complete vaccination schedule. On the other hand, the description of these outcomes was carried out through an active search for COVID-19 cases by the Mitu health secretary.

The disease's severity was defined by the following criteria [9, 10]: A) Mild disease: local symptoms in the upper respiratory tract and may present with non-specific symptoms such as fever, pain muscle, or general discomfort. B) Moderate disease: clinical or radiological evidence of lower respiratory infection, with compatible lung images and O2 saturation > 93%, and C) Severe disease: respiratory rate greater than 30/min, oxygen saturation < 93%, PAFI (the relationship between arterial oxygen pressure and the inspired fraction of oxygen (PaO2 / FIO2) less than 300, infiltrates greater than 50%.

Ethical aspects

The research was carried out following the international ethical standards given by the World Health Organization (WHO) and the Pan American Health Organization, supported by the Declaration of Helsinki and the Ministry of Health of Colombia resolution number 008430 of 1993 and endorsed by the Committee of Ethics of the Institute of Biological Research of the Tropic, University of Córdoba.

Analysis of data

The data were analyzed by the biostatistics group of the Institute of Biological Research of the Tropic-University of Córdoba using the statistical package for the Social Sciences version 27 (SPSS) and the software GraphPad Prisma 8, and univariate analysis was performed. For qualitative variables, it was performed through the calculation of absolute and relative frequencies. The measures of central tendency were calculated as quantitative variables.

Results

Characteristics of the evaluated population

60.4% of the population of the municipality of Mitu is predominantly indigenous. Besides, 99.9% (7849 people) completed their vaccination schedule with two doses of CoronaVac®. Of those vaccinated, 45.3% were women and 54.7% men, the median age was 38 years and 84.6% were under 60 years of age, eight (0.1%) women were pregnant and voluntarily vaccinated (Table 1).

Incidence of SARS-CoV-2 infections after vaccination

From March 23 to August 10, 2021, 447 cases have been presented, corresponding to 5.7% of vaccinated individuals (Table 2). Regarding the severity of the infection,

Fig. 1 The geographic location of the municipality of Mitu. This figure showed that Mitu is located in the southeast of Colombia on the border with Brazil

the age range, under 60 years there were 406 infections, of these 405 (99.8%) were mild infections and one (0.2%) with moderate severity, and in those over 60 years, there were 41, of these 40 (97.6%) were mild infections and one (2.4%) was severe, and this individual died as a direct consequence of COVID-19 (Table 3).

In May 2021, in Mitu, a new peak of SARS-CoV-2 was observed with 200 cases. This increase is much lower than the August 2020 peak, where 327 were reported. In addition, it can be observed that between April–May 2021, the highest peak of individuals who completed their CoronaVac® vaccination reduced COVID-19 cases by 72% in June (Fig. 2). On the other hand, when comparing the fatality rate, it was 2.2% before vaccination and 0.22% in the immunized population (Table 4).

Vaccination effectiveness in the different forms of the severity of COVID-19

Regarding the vaccine's effectiveness, it was observed that it was 94.3% to prevent mild forms and 99.9% for the case of moderate and severe forms. Besides, the vaccine was 99.9% effective in preventing cases of death attributed to SARS-CoV-2 has been reported among the vaccinated group (Table 4).

Discussion

The vaccine demonstrated a significant of 94.3% efficacy in clinical trials for preventing SARS-CoV-2 infections in different stages of severity. With this efficacy, herd immunity may have been achieved through mass vaccination in this population. This vaccine's effectiveness

Table 1 Characteristic of the individuals vaccinated with two doses in Mitu municipality

Characteristic of the individuals vaccinated (%)	
Sex	
Female	3530 (45)
Male	4319 (55)
Median age in years (range)	38 (18–95)
Individuals < 60 years	6644 (84.6)
Individuals > 60 years	1205 (15.4)
Ethnicity	
Indigenous	4745 (60.4)
Afro-Colombian	156 (2)
Other	2948 (37.6)
Pregnant women vaccinated	
Yes	8 (0.1)
Total of people with two doses	7849 (99.9)

study in a predominantly indigenous population is similar in size to the phase III studies conducted in Turkey and Brazil, in which between 7000 and 13,000 participants were evaluated [11].

SARS-CoV-2 infections among those vaccinated were mild, and their management was ambulatory. In addition, it has been seen that vaccination with the immunogen from the pharmaceutical company Sinovac has prevented the appearance of complicated infections and fatal outcomes [12]. These findings are consistent with those reported by phase III studies carried out in Brazil, where it was shown that this vaccine reduces the risk of hospitalization and death between 84 to 100% of

Table 2 Characterization of the SARS-CoV-2 infected individuals post-vaccinated

Characteristic of the individuals infected (%)	
Female	**230 (51.5)**
Male	217 (48.5)
Test used for SARS-CoV-2 diagnostic	
Antigen	268 (60)
RT-qPCR	179 (40)
Severity of COVID-19	
Mild	445 (99.6)
Moderate	1 (0.2)
Severe	1 (0.2)
Type of treatment	
Ambulatory care	445 (99.6)
Hospitalized	2 (0.4)
Deceased by COVID-19	1 (0.2)
Total of people infected with COVID-19	447 (5.7)

Table 3 Severity of COVID-19 in population vaccinated according to age range < 60 years vs > 60 years

Severity of COVID-19 according to age range (%)	
< 60 years	***N* = 406**
Mild	405 (99.8)
Moderate	1 (0.2)
Severe	0
> 60 years	*N* = 41
Mild	40 (97.6)
Moderate	0
Severe + deceased	1 (2.4)

individuals vaccinated with CoronaVac® [12]. However, our results in the older than 60 years show differences with what was published in Brazilian older adults by Ranzani et al. [13], who found protection of 49.4%. The vaccine's reduction could be explained because 83% of their cases were infected with the P.1 variant of SARS-CoV-2.

Furthermore, it is essential to analyze the course of infection over time and the impact of vaccination against SARS-CoV-2. In April 2021, the third wave of COVID-19 cases began in Colombia. However, the incidence was much lower than observed in the first peak of the pandemic between April and June 2020. The new cases presented in 2021 in the vaccinated population could be due to the Brazilian variant P.1 of SARS-CoV-2 [14]. However, the morbidity and mortality of this new variant seem to be controlled with the CoronaVac® vaccine.

Regarding the effectiveness of this vaccine, it was observed that it was 94.3% against mild disease and 99.9% against severe infection in this population. Our findings are similar to Turkey's phase III study for CoronaVac®, in which efficacy of 91% was observed. In contrast to studies in Brazil and Chile, which reported low overall efficacy of 50.38 and 65%, respectively. However, it is essential to highlight that this vaccine reduced 90% of the proportion of hospitalization in an intensive care unit (ICU) and 86%mortality from SARS-CoV-2 [15, 16] in the Chilean population. The epidemiological moments of vaccination must also be taken into account. For example, Chile began vaccination with a low viral transmission different from the epidemiological scenario studied in Brazil. When the transmission is lower, there is less chance that vaccination will fail [17]. Our study is similar to perform in the small city of Serrana, Brazil, that vaccinated using CoronaVac®. In Serrana, 95% of the city's adult population was vaccinated, a reduction of 80% in symptomatic cases and hospitalizations dropped by 86% and mortality by 95% [5].

So far, SARS-CoV-2 is a virus that is efficiently transmitted and quickly infects the unvaccinated population.

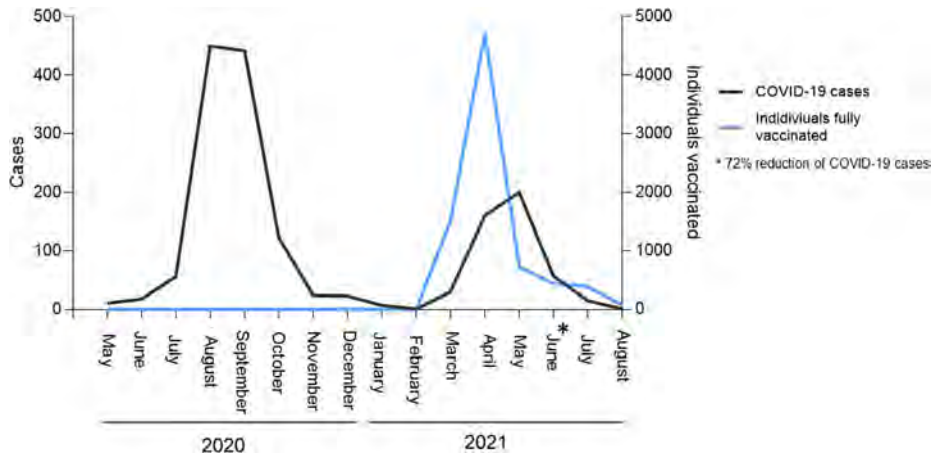


Fig. 2 Characterization of COVID-19 cases in Mitu municipality. This figure showed a first peak or wave of cases of COVID-19 in August 2020, with a significant drop of cases in November 2020. The vaccination in this municipality started in February 2021 and a second wave was observed between April–May 2021, the highest peak of individuals who completed their CoronaVac® vaccination reduced COVID-19 cases by 72% in June

Due to the lack of genotypic information for the Mitú municipality, we do not know if the P1 variant (Brazil) managed to spread or if the action of the vaccine contained it. On the other hand, one of the limitations of this work could be in a possible under-registration of the mild infections registered in this vaccine population, since it was not possible due to the type of study that was proposed to carry out a strict follow-up by RT-qPCR to this population cluster.

The primary outcome of this study was to evaluate the effectiveness of CoronaVac® in reducing mortality and severe illness due to SARS-CoV-2. On the other hand, one of the limitations of this work could be in a possible under-registration of the mild infections registered in this vaccine population, since it was not possible due to the type of study that was proposed to carry out a strict follow-up by RT- qPCR to this population cluster.

Finally, we can infer that to date, herd immunity has been achieved through mass vaccination in this population, which has impacted the reduction of complicated cases and the mortality rate from COVID-19. However, pediatric populations remain unvaccinated, which could cause few breakthrough infections with an increase in the number of cases at a given epidemiological moment. It is also necessary to know if the CoronaVac® will protect against the new delta strain in Colombia. It will be a real challenge for the vaccine in a couple of months when it is believed that Delta could be predominant in Colombia. Public health must continue long-term surveillance to measure the effect of vaccination in the studied population. It is unknown if the vaccine's immunity will be maintained over time and if a booster of this immunogen is needed in the short or medium term. There is still a long way to walk on this exciting research topic that will be key to controlling and mitigating the pandemic caused by SARS-CoV-2.

Table 4 Effectiveness of the Coronavac vaccine

Effectiveness of CoronaVac	
Prevent mild forms	**94.3%**
Prevent moderate forms	99.9%
Prevent severe forms	99.9%
Prevent deaths	99.9%
Mortality rate pre-vaccination*	2.2%
Mortality rate post-vaccination in individuals fully vaccinated	0.22%

*Data obtained from DANE Colombia. (https://www.dane.gov.co/files/investigaciones/poblacion/defunciones-covid19/boletin-defunciones-covid-2020-02mar-2021-17ene.pdf)

Abbreviations
COVID-19: Coronavirus Disease 2019; WHO: World Health Organization; ICU: Intensive Care Unit

Acknowledgments
To Ministerio de Ciencia Tecnología e Innovación, Colombia (MINCIENCIAS), Secretary of Health of Mitu, Vaupes.

Authors' contributions
Study design, SM, HM; **Data collection**, HM, JMR, SG, **Methodology**, RR, KG, BG. **Data analysis, curation and interpretation**, HSC, SM; **Writing / Drafting,** HSC, SM; **Critical revision of the article**, LHP, CG, JM, BG, RR. All authors have read and agreed to the published version of the manuscript.

Funding
Any organization did not fund our project.

Availability of data and materials
The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

Declarations

Ethical approval and consent to participate
The research was carried out following the international ethical standards given by the WHO and the Pan American Health Organization, supported by

the Declaration of Helsinki, and national legislation, resolution number 008430 of 1993 of the Ministry of Health of Colombia that regulates the studies in health. Furthermore, this work was endorsed by the ethics committee of the Tropic Biological Research Institute.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Universidad de Córdoba, Instituto de Investigaciones Biológicas del Trópico, Montería, Colombia. [2]Instituto Colombiano de Medicina Tropical-Universidad CES, Medellín, Colombia. [3]Secretaria de Salud del Vaupés, Mitú, Colombia.

Received: 7 June 2021 Accepted: 2 November 2021
Published online: 15 January 2022

References

1. World Health Organization. Coronavirus Disease (COVID-19) Dashboard [Internet]. 2021. Available from: https://covid19.who.int/
2. Our World in Data. Coronavirus (COVID-19) Vaccinations [Internet]. 2021. Available from: https://ourworldindata.org/covid-vaccinations
3. Sabino EC, Buss LF, Carvalho MPS, Prete CA, Crispim MAE, Fraiji NA, et al. Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. Lancet. 2021;397(10273):452–5. https://doi.org/10.1016/S0140-6736(21)00183-5.
4. Liu R, Munroe R, Sarkar TH. China Sinovac says it reached two billion doses annual capacity for COVID-19 vaccine | Reuters [Internet]. Reuters. 2021 [cited 2021 Dec 21]. Available from: https://www.reuters.com/article/us-health-coronavirus-vaccine-sinovac-idUSKBN2BP07G.
5. Mallapaty S. WHO approval of Chinese CoronaVac COVID vaccine will be crucial to curbing pandemic [internet]. 2021. Available from: https://www.nature.com/articles/d41586-021-01497-8
6. Ministerio de Salud Colombia. Plan Nacional de Vacunación contra COVID-19 [Internet]. 2021. Available from: https://app.powerbi.com/view?r=eyJrIjoiNThmZTJmZWYtOWFhMy00OGE1LWFiNDAtMTJmYjM0NDA5NGY2IiwidCI6ImJmYjdlMTNhLTdmYjctNDAxNi04MzBjLWQzNzE2ZThkZDhiOCJ9
7. Palacios R, Patiño EG, de Oliveira PR, Conde MTRP, Batista AP, Zeng G, et al. Double-blind, randomized, placebo-controlled phase III clinical trial to evaluate the efficacy and safety of treating healthcare professionals with the adsorbed COVID-19 (inactivated) vaccine manufactured by Sinovac - PROFISCOV: a structured summary of a study protocol for a randomised controlled trial. Trials. 2020;21(1):853. https://doi.org/10.1186/s13063-020-04775-4.
8. Wu Z, Hu Y, Xu M, Chen Z, Yang W, Jiang Z, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021;3(6):803–12. https://doi.org/10.1016/S1473-3099(20)30987-7.
9. Saavedra TC. Resumen: Consenso colombiano de atención, diagnóstico y manejo de la infección por SARS-COV-2/COVID-19 en establecimientos de atención de la salud Recomendaciones basadas en consenso de expertos e informadas en la evidencia [Internet]. 2020. Available from: http://www.revistainfectio.org/index.php/infectio/article/view/851/896.
10. World Health Organization. Clinical management of COVID-19 [Internet]. 2020. Available from: https://www.who.int/publications/i/item/clinical-management-of-covid-19
11. Haidere MF, Ratan ZA, Nowroz S, Zaman SB, Jung Y-J, Hosseinzadeh H, et al. COVID-19 vaccine: critical questions with complicated answers. Biomol Ther (Seoul). 2021;29(1):1–10. https://doi.org/10.4062/biomolther.2020.178.
12. Sinovac Biotech Ltd. Sinovac Announces Phase III Results of Its COVID-19 Vaccine [Internet]. 2021 [cited 2021 Dec 21]. Available from: http://www.sinovacbio.com/news/shownews.php?id=1152&lang=en.
13. Ranzani OT, Hitchings MDT, Dorion M, D'Agostini TL, De Paula RC, De Paula OFP, et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: Test negative case-control study. BMJ [Internet]. 2021 Aug 20 [cited 2021 Dec 21];374:2015. Available from: https://www.bmj.com/content/374/bmj.n2015.
14. Kupferschmidt K. New mutations raise specter of "immune escape". Science. 2021;371(6527):329–30. https://doi.org/10.1126/science.371.6527.329.
15. He Q, Mao Q, Zhang J, Bian L, Gao F, Wang J, et al. COVID-19 vaccines: current understanding on immunogenicity, safety, and further considerations. Front Immunol. 2021;12:669339. https://doi.org/10.3389/fimmu.2021.669339.
16. Ministerio de Salud-Chile. Reporte COVID-19: Vacuna CoronaVac tiene un 90,3% de efectividad para prevenir el ingreso a UCI [Internet]. 2021. Available from: https://www.minsal.cl/reporte-covid-19-vacuna-coronavac-tiene-un-903-de-efectividad-para-prevenir-el-ingreso-a-uci/
17. Urwicz T. Asesor del Ministerio de Salud de Chile: "El virus se adaptó para transmitirse rápidamente" [Internet]. 2021. Available from: https://www.elpais.com.uy/informacion/salud/asesor-ministerio-salud-chile-virus-adapto-transmitirse-rapidamente.html?utm_source=EPD&utm_medium=Push&utm_campaign=Notificaciones#pk_campaign=MASwpn&pk_kwd=%22El+virus+se+adapt%C3%B3+para+transmitirse+r%C3%A1pidamente%22.

Publisher's Note

CoronaVac

O que a ciência comprova

1.7. Estudo de Serrana mostra efetividade de 80,5% da CoronaVac contra casos de Covid-19 e 94,9% contra mortes; vacinação protegeu inclusive não vacinados contra a variante gama

Os dados da primeira análise do Projeto S, estudo de efetividade da vacina CoronaVac que o Butantan conduziu no município paulista de Serrana, mostram uma efetividade direta de 80,5% contra casos sintomáticos de Covid-19, de 95% contra hospitalizações e de 94,9% contra mortes. A pesquisa também indica que, com 52% da população vacinada, os efeitos indiretos começam a se manifestar, protegendo inclusive quem não tomou o imunizante. Além disso, na época do estudo (entre fevereiro e maio de 2021), a maioria dos casos eram provocados pela variante gama (P.1, amazônica) do SARS-CoV-2, o que evidencia novamente que a CoronaVac é eficaz contra essa cepa – que predominou no Brasil em todo o primeiro semestre de 2021.

Os resultados da pesquisa, conduzida por cientistas do Instituto Butantan, do Hospital Estadual de Serrana, da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo e da Secretaria Municipal da Saúde de Serrana, estão descritos no artigo "Projeto S: a stepped-wedge randomized trial to assess CoronaVac effectiveness in Serrana, Brazil", divulgado na plataforma de preprints SSRN.

O Projeto S – um ensaio clínico do tipo randomizado escalonado – é o primeiro estudo clínico controlado que demonstra a eficiência de um imunizante no mundo real e seu efeito indireto na população não vacinada, tendo sido realizado durante uma pandemia e sem utilizar grupo controle. A pesquisa é pioneira ao demonstrar que uma vacina de vírus inativado utilizada como medida de emergência de saúde pública primária pode mudar o curso de uma epidemia. Além disso, o estudo mostra que as vacinas são o pilar para conter o número de casos e a transmissão viral e para controlar os efeitos devastadores da Covid-19.

Os voluntários do Projeto S foram vacinados com a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, em um esquema de duas doses com um intervalo de quatro semanas. No total, completaram o esquema vacinal 81,3% da população adulta

e 60,9% da população urbana de Serrana, o equivalente a cerca de 27 mil pessoas. Deste número, 16% eram idosos com mais de 60 anos.

A eficácia geral da vacina foi estimada comparando a incidência de casos pré e pós-vacinação para toda a população urbana. A eficácia direta foi avaliada na relação entre a incidência de casos em indivíduos totalmente vacinados e não vacinados. Entre os vacinados, a efetividade direta da vacina foi de 80,5% (IC 95%, 75,1 a 84,7) na prevenção de casos sintomáticos; de 95% (IC 95%, 86,9 a 98,1) contra hospitalizações; e de 94,9% (IC 95%, 76,4 a 98,9) para prevenir mortes. Durante o período do estudo, 1.447 casos de Covid-19 foram reportados em Serrana; destes, 361 (24,9%) foram sequenciados, indicando uma incidência da variante gama de 92% a 100% na cidade.

Ao analisar o impacto da vacinação em maiores de 60 anos, a efetividade direta da CoronaVac permanece muito alta: 86,4% (IC 95%, 74,5 a 93) na prevenção de casos sintomáticos, 96,9% (IC 95%, 86,1 a 99,3) contra hospitalizações e 96,9% (IC 95%, 73,9 a 99,6) para prevenir mortes.

Os pesquisadores salientam que não é possível fixar um nível mínimo de imunização para controlar a Covid-19 em uma área, mas que os resultados do Projeto S demonstram que quando 52% da população havia recebido as duas doses da vacina, os efeitos indiretos de proteção começaram a ser observados nos outros grupos que ainda não haviam completado a imunização – sugerindo um indicador de imunização para controlar a variante gama do SARS-CoV-2. Além disso, durante o período do estudo, o número de infecções entre crianças também foi reduzido, indicando o efeito indireto da CoronaVac nesta população, que não foi imunizada. No entanto, relatam que os efeitos diretos da vacinação foram superiores aos indiretos, reforçando a necessidade de se vacinar o maior número possível de pessoas rapidamente.

Publicado em: 29/11/2021

Artigo

Original Research

Title: Projeto S: a stepped-wedge randomized trial to assess CoronaVac effectiveness in Serrana, Brazil

Marcos Carvalho Borges, M.D., Ph.D.*[1,2&]

Ricardo Palacios, M.D., Ph.D.[3&]

Hugo Alberto Brango[3]

Mônica Tilli Reis Pessoa Conde, M.D., Ph.D.[3]

Elizabeth González Patiño[3]

Ana Paula Batista[3]

Barbara Marques Coutinho[2]

Gilberto Guedes de Padua[3]

Glenda Renata de Moraes[4]

Gustavo Jardim Volpe, M.D., Ph.D.[2]

Joane do Prado Santos[3]

Natasha Nicos Ferreira, M.D.[2]

Pedro Manoel Marques Garibaldi, M.D.[2]

Ricardo Haddad, Ph.D.[3]

Roberta de Oliveira Piorelli[3]

Sandra Coccuzzo Sampaio Vessoni, Ph.D.[3]

Simone Kashima Haddad[1]

Benedito Antônio Lopes Fonseca, M.D., Ph.D.[1]

Rodrigo Tocantins Calado, M.D., Ph.D.[1]

Dimas Tadeu Covas, M.D., Ph.D.[1, 3]

* Corresponding author: Marcos Carvalho Borges (marcosborges@fmrp.usp.br)

[1] Ribeirão Preto Medical School, University of São Paulo, São Paulo, SP, Brazil
[2] Serrana State Hospital, SP, Brazil
[3] Instituto Butantan, São Paulo, SP, Brazil
[4] Health Department, Serrana, SP, Brazil

[&]Drs. Marcos Carvalho Borges and Ricardo Palacios contributed equally to this article

Abstract

Background:

A stepped-wedge trial is an approach for assessing vaccine effectiveness in the real world. By the end of the study, all participants could receive the intervention, eliminating the ethical dilemma of placebo, especially during a pandemic.

Methods:

We evaluated the effectiveness of CoronaVac in Serrana, Brazil, amid an uncontrolled community Covid-19 epidemic using a stepped-wedge randomized trial. The city was separated into 25 subareas, divided into four groups, and randomized to receive CoronaVac in a two-dose scheme with a four-week interval. Intervention was initiated in each group with a one-week interval. The primary endpoint was the incidence of symptomatic cases in fully immunized individuals. The secondary endpoints were Covid-19-related hospitalizations and deaths and incidence according to immunization coverage.

Findings:

The study occurred during epidemiological weeks 6 to 19 in 2021. Up to 27,406 participants received the first dose of the study vaccine, corresponding to 81·3% of the adults and 60·9% of the urban population. Among fully immunized individuals, the vaccine effectiveness was 80·5 (95% CI, 75·1 to 84·7) for preventing symptomatic Covid-19 cases, 95% (95% CI, 86·9 to 98·1) and 94·9% (95% CI, 76·4 to 98·9) for preventing Covid-19-related hospitalizations and deaths, respectively. There was a significant indirect protective effect in unvaccinated people when 52% of the adult population was fully vaccinated. The Gamma variant was dominant during the study.

Interpretation:

CoronaVac effectively prevented symptomatic Covid-19 cases and protected against severe disease and death during Gamma variant circulation. Unvaccinated individuals benefited from high vaccine coverage levels.

(ClinicalTrials.gov Identifier, NCT04747821)

Funding

Fundação Butantan and São Paulo Research Foundation (FAPESP).

Introduction

The ongoing Coronavirus Disease 2019 (Covid-19) pandemic has an unprecedented burden in modern times in loss of lives, people living with sequelae, and increased poverty.[1] Covid-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, is associated with a broad spectrum of clinical manifestations ranging from mild symptoms to death.[2,3]

Among the measures to control disease's devastating effects, vaccines have been proposed as a cornerstone to curb the number of cases and viral transmission. In December 2020, the first vaccine was approved in the United Kingdom,[4] and in mid-January 2021, two vaccines, inactivated SARS-CoV-2 vaccine (CoronaVac) and ChAdOx1 nCoV-19 vaccine (Oxford–AstraZeneca), were approved for emergency use in Brazil.[4-6]

Although currently approved vaccines have shown efficacy in randomized studies, phase 3 trials have limitations and do not demonstrate vaccine effectiveness, such as reduction in hospitalizations and deaths or decrease in virus transmission.[7,8] Investigation of effectiveness in real world is challenging but highly relevant, especially in vaccine scarcity conditions.

In the 1980s, the stepped-wedge trial design was proposed to assess the effectiveness of the Hepatitis B vaccine allowing all communities to eventually get access to immunization.[9] More recently, this study design was proposed as an ethical approach for assessing vaccine effectiveness during the Ebola emergency, but it was never carried out because of the decrease in case incidence.[10,11]

The lack of a placebo group in stepped-wedge trials allows all participants to receive the intervention at the end of the study, eliminating the ethical dilemma of placebo, especially during a pandemic. Since the intervention occurs at different periods, group comparisons can be made between, as well as a broad analysis before and after intervention. In contrast to mass

vaccination, the indirect protective effect of vaccination also can be assessed in a stepped-wedge trial. [10-12]

In the present study, we used a stepped-wedge randomized trial to assess the effectiveness of an inactivated Covid-19 vaccine in an entire city in Brazil during the uncontrolled regional Covid-19 epidemic.

Methods

Study design and participants

This study is a stepped-wedge randomized trial conducted in Serrana, one of the 26 municipalities of the Regional Health Department XIII in the State of São Paulo in Brazil. Each day, a quarter of the population commute to nearby cities, such as Ribeirão Preto, facilitating the transmission of infectious diseases.

The estimated population for 2020 was 44,434 inhabitants, according to the Statistical Website of the State of São Paulo (populacao.seade.gov.br), which was based on an official and compulsory census conducted in 2010 (Table 1). Adults aged 18 years and over residing in the city were eligible for the study. A list of all inclusion and exclusion criteria are provided in the appendix.

First, the city administration, Housing and Urban Development Company, Serrana State Hospital, the Butantan Institute, and local workers created a city participatory mapping and the urban region was divided into 25 subareas, according to the land use.[13] Next, the 25 urban subareas were reassembled in four color-coded groups (Green, Yellow, Gray and Blue), balancing population among groups and avoiding contiguous areas coded with the same color (Figure S1). The subareas were reassembled into the groups by an investigator (RP) who was not involved in the mapping nor had links with the city.

The study was reviewed and approved by the Ethics Committee of the Clinical Hospital, Ribeirão Preto Medical School, University of São Paulo (CAAE 42390621.1.0000.5440). The study is registered on ClinicalTrials.gov (NCT04747821).

Randomization

The study was presented to the community on February 6, 2021 in a public venue with support from local authorities and leaders. During the event, intervention order for the groups was determined in a public draw. The randomized order was Green, Yellow, Gray, and Blue. Vaccination occurred in each color-coded group with one-week intervals (Figure 1).

Procedures

Eight public schools were adapted as study subsites where potential participants were assessed for eligibility, including confirmation of residential address and if the area was suitable for recruitment at that week, and were consented. All participants had blood drawn to assess the presence of antibodies against SARS-CoV-2 by using Elecsys anti-SARS-CoV-2 and Elecsys anti-SARS-CoV-2 S (*Roche Diagnostics*), according to the manufacturer's instructions, and test for pregnancy in women of childbearing age.

Participants were vaccinated with CoronaVac (*Sinovac Life Sciences, Beijing, PRC*), an inactivated Covid-19 vaccine, in a two-dose scheme with four-week interval, from a single lot (#202009004). Participants who missed vaccination were rescheduled within one week. Vaccination subsites were open from Wednesday to Sunday between February 14 and April 11, 2021.

All participants stayed for half-hour after vaccination under medical supervision. Participants were advised to seek medical attention at local healthcare units, which reported all cases of adverse events within seven days after immunization. During the study period,

vaccination was allowed by the National Immunization Program, which definition is provided in the appendix.

Since September 2020, there has been enhanced case surveillance for Covid-19 cases in Serrana. Any person with one or more symptoms (cough, fever, muscle pain, headache, nausea, vomiting, diarrhea, dysgeusia, anosmia, dyspnea, coryza, nasal congestion, sore throat, or fatigue) for at least two days had access to any of the local healthcare units of the municipality and was tested for free for SARS-CoV-2 by RT-PCR nasal swab. Results were available the next working day. Positive samples for SARS-CoV-2 during the study period were analyzed and sequenced for variant detection. The study surveillance started the day after randomization (epidemiological week 6). The case initial date considered for analysis was the day of the beginning of symptoms. Patients were followed for 28 days or until hospital discharge or death. Safety surveillance focused on medically attended adverse reactions.

All cases reported by the Serrana health authorities or from other cities in public health surveillance systems (e-SUS and SIVEP-Gripe) as residing in Serrana were included in the analysis. Those systems also were used to collect information from cases residing in other municipalities of the Regional Health Department XIII.

Outcomes

The primary analysis units were the color-coded groups, which were used for allocation. Color-coded groups were randomized to receive vaccination at one-week intervals (Figure 1). The adult population (18 years or older) residing in each corresponding group was invited to join the study in the corresponding week. Only urban areas were considered for the study analysis, corresponding to 91·4% of the population (44,183); however, the study

vaccine also was offered to residents in rural areas of the municipality, including those in permanent and temporary settlements.

The study analysis comprehended from epidemiological weeks 6 to 19 in 2021 and involved three study periods for each color-coded group: Control period, before vaccination; Transition period, from first vaccination up to six weeks later; and Intervention period, starting six weeks after initial dose (when participants are expected have two weeks or more after full vaccination scheme) to epidemiological week 19 (Figure 1).

The primary endpoint was the incidence of symptomatic Covid-19 cases in fully immunized individuals. Secondary endpoints included the incidence of Covid-19-related hospitalizations and deaths, incidence of cases according to immunization coverage, change in the number of cases in comparison to neighboring cities, and frequency of SARS-CoV-2 variants.

Statistical analysis

Information from study participants and case and safety surveillance were cross-checked to determine the area and status regarding the intervention. To calculate vaccine effectiveness, case incidence was first determined using a mixed *Poisson* regression model to verify weekly changes in incidence rate ratios (IRR). Let y_{ij} be the number of Covid-19 cases in the group i ($i = 1,2,3,4$) during the epidemiological week j ($j = 6, \dots ,19$).

The model is written as follows:

$$y_{ij} = \mu + \alpha_i + \theta X_{ij} + \varepsilon_{ij}$$

Here, μ is the baseline rate, $\alpha_i \sim N(0,\sigma_\alpha^2)$ is a random effect for the group i , X_{ij} represents the interventional group status i during epidemiological week j and $\varepsilon_{ij} \sim N(0,\sigma_\varepsilon^2)$. We categorized the treatment variable according to vaccination status, where the epidemiological weeks 6 and 7 were assumed as reference, so that θ represents the gradual effect of the intervention.

After case incidence estimation, vaccine effectiveness was calculated using two different methods: overall effectiveness and direct vaccine effectiveness.

The overall effectiveness was estimated by comparing the case incidence for the entire urban population in the control vs. the intervention period, as 100×(1–IRR) and 95% CIs for vaccine effectiveness estimated as 100×(1–upper or lower bounds of 95% CI for IRR), where:

$$IRR = \frac{(number\ cases_{intervention\ period})/(total\ person-days\ at\ risk_{intervention\ period})}{(number\ cases_{control\ period})/(total\ person-days\ at\ risk_{control\ period})},$$

and the 95%CI for IRR was calculated as, $e^{\{log(IRR)\ \pm\ 1{\cdot}96\ \times\ SE(log(IRR))\}}$ with the standard error for $log(IRR)$:

$$SE(log(IRR)) = \sqrt{\frac{1}{number\ cases_{intervention\ period}} + \frac{1}{number\ cases_{control\ period}}}.$$

The direct vaccine effectiveness (dVE) was calculated by comparing the incidence density between fully vaccinated and unvaccinated participants during intervention period as follows:

$$dVE = 1 - \frac{(cases_{Vaccinated})/(Total\ person-days\ at\ risk_{Vaccinated})}{(cases_{Unvaccinated})/(Total\ person-days\ at\ risk_{Unvaccinated})}.$$

Indirect protective effect was determined combining two parameters. First, it was determined the epidemiological week when a significant and persistent decrease in case incidence occurred for the entire population. Second, the epidemiological week when an anticipated effect was observed in a color-coded group, i.e., when a significant reduction in the case incidence occurred before the sixth week after the second vaccine dose. After defining the epidemiological week that indirect protective effect occurred, the respective vaccine coverage was defined.

The cumulative incidence for Covid-19-related hospitalization and death for Serrana and the other nearby municipalities from Regional Health Department XIII was calculated and compared between epidemiological weeks 6 and 19.

Role of the funding source

The study was supported by the Fundação Butantan, a non-profit foundation supporting activities of the Instituto Butantan, a public health research institution of the Government of São Paulo State, and by the São Paulo Research Foundation (FAPESP, grant 2020/10127-1). The vaccine manufacturer, Sinovac Life Sciences, had no role in the study but provided the product at no cost.

Results

Between Feb 14, 2021, and April 11, 2021, 28,656 individuals gave written informed consent and were enrolled in the study, 908 were excluded before vaccination mainly due to unstable chronic disease, treatment with immunosuppressive therapy, impaired immune system diseases and alcohol or drug abuse, and 27,748 participants received the first vaccine dose. Also, 342 individuals were excluded from the study analysis because they lived in rural areas. Thus, 27,406 residents in urban areas received the first dose, corresponding to 82·9% of the adults and 62% of the estimated urban populations. Only 515 (1·9%) participants did not receive the second dose mainly due to Covid-19-related symptoms, treatment with immunosuppressive therapy, and pregnancy. Thus, 81·3% of the adults and 60·9% of the overall urban population completed the vaccination scheme.

The participant distribution by gender was comparable (50·4% female), and 16% of the participants were 60 years or older. Before vaccination antibodies against nucleocapsid

and receptor-binding domain (RBD) were detected in 23·6% and 24·6% of participants, respectively. The baseline details per color-coded group are summarized in Table 1.

The number of symptomatic Covid-19 cases detected during the study period was 1,447. Of these, 149 resulted in hospitalization or death. In cases with reported symptoms between epidemiological weeks 6 and 19, there were 37 fatalities. The cumulative incidence of symptomatic and hospitalization cases is depicted in Figure S2.

The overall vaccine effectiveness for the whole population, including vaccinated and unvaccinated people, was 48·1% (95% CI, 39·2 to 55·7) for preventing symptomatic Covid-19 cases and 48·1% (95% CI, 13·2 to 69·0) for preventing disease-related hospitalization or death. Overall vaccine effectiveness according to study period and age is shown in Figure S3. Among fully immunized individuals, the direct vaccine effectiveness was 80·5 (95% CI, 75·1 to 84·7) for preventing symptomatic Covid-19 and 95% (95% CI, 86·9 to 98·1) and 94·9% (95% CI, 76·4 to 98·9) for preventing Covid-related hospitalization and death, respectively (Table 2). A significant direct vaccine effectiveness in the elderly has been shown in Table 2.

Out of the 1,447 reported Covid-19 cases, 361 (24·9%) samples were completely sequenced during the study period. The Gamma variant accounted for 92% to 100% of the circulating lineage between epidemiological weeks 10 and 19. Moreover, other lineages were also detected, demonstrating the replacement of the ancestral lineage (Figure S4).

The analytical model revealed a significant increase in the IRRs in epidemiological week 10 when the Blue group received the first dose (1·59, $p<0·001$). This tendency was reverted by epidemiological week 13 (0·58, $p<0·001$). A significant indirect protective effect was observed in epidemiological week 13, when the adult population coverage reached 52%. Notably, the maximum decrease in case incidence occurred by week 15 (0·25, $p<0·001$), which corresponds to one week after Blue group received the second dose, and remained low until the end of the experimental period (Figure 2 and Table S2).

Concerning hospitalization and death, the peak number of cases occurred in week 10 (2·00, p=0·02), and a maximum decrease was found on week 15 (0·17, p=0·02). For the remainder of the study, the hospitalization and death case numbers remained low and insignificant due to the small sample size (Figure 2 and Table S2).

Assessments of the IRRs for the symptomatic Covid-19 cases of each group were performed in a chronological sequence (Figure 2). The Green group, vaccinated between weeks 7 and 11, exhibited a significant decrease in the IRR, beginning at week 14 (0·32, p<0·001). In the Yellow group, vaccinated between weeks 8 and 12, a reduction in the IRR was detected at week 14 (0·35, p=0·046). The Gray group, vaccinated on weeks 9 and 13, displayed significant attenuation of the IRR at week 15 (0·30, p=0·049). In the Blue group, vaccinated between weeks 10 and 14, the IRR reduction was detected as early as at week 13 (0·15, p<0·001), one week earlier than the previous group, demonstrating the indirect protective effect of vaccination. The model cannot be adjusted for hospitalizations and deaths due to the limited number of cases (Figure S5).

From epidemiological weeks 6 to 13, the cumulative incidence for Covid-19-related hospitalization and death in Serrana overlapped with other cities in the region. However, this scenario changed during epidemiological week 13 when the incidence in Serrana was deterred, whereas in other cities in the region it remained high (Figure 3).

Discussion

In the context of a public health emergency, this is the first study to demonstrate how a vaccine can change the course of an ongoing epidemic in a region with no other significant measures. Among fully immunized individuals, CoronaVac proved effective at preventing symptomatic Covid-19 cases and disease-related hospitalization and death in adults and

elderly. Notably, the stepped-wedge experimental design confirmed the collective immunity and the indirect protective effect of community vaccination.

Notably, our study demonstrated a direct vaccine effectiveness of 80·5 (95% CI, 75·1 to 84·7) for symptomatic SARS-CoV-2 infection when the Gamma variant was predominant. A Chilean study reported vaccine effectiveness of 65·9% for symptomatic Covid-19 and 87·5% and 86·3% for disease-related hospitalization and death, respectively, using administrative observational data from a mass vaccination campaign.[14] It should be pointed out that in Chile the population was vaccinated over four months, whereas in Serrana the immunization was performed in two months. Since the stepped-wedge strategy produced results consistent with data obtained from a larger study, it should be considered a practical approach for assessing and predicting the real-world performance of new vaccines.

Nonetheless, in a previous test-negative case-control study that enrolled healthcare workers in Manaus, Brazil, CoronaVac effectiveness was found to be 49·6% (95% CI, 11·3 to 71·4) after the first dose and 36·8% (95% CI, -54·9 to 74·2) after the second dose against symptomatic cases.[15] The attenuated effectiveness observed in Manaus could be attributed to study design differences and higher viral exposure. Our results reinforce the importance of immunization as a collective public health measure.

Uncontrolled studies have evaluated the effectiveness of different vaccines, mainly in high-income countries, using the BNT162b2 messenger RNA (mRNA) vaccine (Pfizer–BioNTech), the ChAdOx1 nCoV-19 vaccine (Oxford–AstraZeneca), and the mRNA-1273 vaccine (Moderna).[16-18] Although phase-3 clinical trials of CoronaVac have demonstrated an efficacy ranging from 50·7% in Brazil to 83·5% in Turkey,[5,6] up to now, this is the first controlled clinical study proving its effectiveness in the real world.

CoronaVac is known to have good efficacy in two weeks after complete immunization and, like other Covid-19 vaccines, does not trigger sterilizing immunity. Herein, we reported

that the groups vaccinated later in the experimental period attained the expected effectiveness even before completion of the immunization scheme, indicative of an indirect protective effect. Furthermore, the overall Covid-19 incidence was deterred in Serrana, in contrast with the persistent increase of cases in nearby cities. We also observed in the Intervention period a reversal in the increased trend of symptomatic SARS-CoV-2 cases among children (Figure S3), which would suggest an indirect protective effect of vaccination.

The indirect benefits of other vaccines have already been demonstrated and calculated.[19] Concerning Covid-19 vaccines, mechanisms for indirect effects, such as reduced viral load in respiratory fluids and faster viral clearance, have been proposed.[20] The results of our study found clear indication of indirect protective effects on the unvaccinated population, but the direct vaccine effect is far more important and all efforts should keep focusing on increasing immunization coverage.

Of note, vaccination acceptance was high in all study areas, and the distribution of the stepped-wedge vaccination groups was uniform in the territory. This homogeneity is critical since an unbalanced distribution of vaccination coverage can lead to one or more highly transmissible foci and prevent broader disease control. This study cannot ascertain a minimum immunization level to control the disease throughout the entire territory. However, our results demonstrated that when 52% of the whole population was fully vaccinated, indirect protective effects were observed, suggesting that this might be the minimum level of immunization needed to be achieved for the Gamma variant.

Considering that viral replication might change, it is advisable to make additional efforts to reach immunization levels as high as possible, especially in communities with reduced access to health systems. The ideal vaccination coverage might vary according to SARS-CoV-2 variant transmissibility and adherence to non-pharmacological measures. Unfortunately, this study did not assess if mask use, social distancing and other control

measures changed during and after the experimental period. However, it should be pointed out that Serrana authorities did not promote Covid-19 sanitary measures different from the surrounding region or restrict commuting at any moment.

Like the present study, stepped-wedge clinical trials can provide information about vaccine effectiveness and build confidence in introducing a new immunization scheme. We strongly encourage the inclusion of demonstration studies into the clinical development plan of new vaccines to ease their introduction at a larger scale.[21] In the current case, early results obtained in this trial were vital for boosting CoronaVac's credibility in a scenario of disinformation propagated by public figures.[22] Close coordination between researchers, local and state authorities, and community leaders was critical for making this study possible, and it was reflected in the high vaccine acceptance. The role of community leaders in promoting the study immunization program was also an essential aspect of successful immunization.

Our study has limitations. First, due to the relatively short follow-up, we cannot extrapolate data for late outcomes, such as the duration of the vaccine protection. Second, as the number of severe patients was quite low, the statistical model for the indirect effect could not be adjusted for hospitalizations and deaths per group. Finally, if the rate of infection was trending down in Serrana, the calculated effectiveness could be biased. However, as the study period was relatively short and the case incidence in the nearby cities increased during the study period and in the following months, this stepped-wedge potential bias is unlikely to change the magnitude of our findings.[10]

In conclusion, this study demonstrates that collective immunization can increase Covid-19 vaccine effectiveness. Even in a scenario with new SARS-CoV-2 variant and in areas where very high transmission occurred, the direct and indirect effects of CoronaVac were remarkable. All the approved Covid-19 vaccines are expected to trigger collective

immunity, but each might have different immunization coverage to achieve this effect. Nonetheless, our study provided a proof-of-concept for Covid-19 control through vaccination.

Contributors

RP conceived this study. MCB, HAB, MTRPC, EGP, APB, GGP, GJV, NNF, PMMG, RH, ROP, and DC contributed to the trial design and protocol. MCB is the principal investigator, performed research, and coordinated the study. RP and MCB drafted the manuscript. GGP, GJV, NNF, PMMG, and RH coordinated the study. MCB, BMC, GGP, GJV, GRM, NNF, PMMG, and RH were involved in the acquisition of data. MCB, RP, HAB, MTRPC, EGP, BMC, GRM, GJV, JPS, NNF, PMMG, RH, ROP, SCSV, SKH, BALF, RTC, DC contributed to the analysis and interpretation of the data. MCB, RP, HAB, MTRPC, EGP, BMC, GJV, NNF, PMMG, ROP, SCSV, SKH, BALF, RTC, DC edited the manuscript. HAB and EGP did the statistical analysis. All authors critically reviewed the manuscript and approved the final version. All authors had full access to all data in the studies and had final responsibility for the decision to submit for publication.

Data sharing

Anonymous participant data will be available upon completion of clinical trials and publication of the results of the completed study upon request to the corresponding author. Proposals will be reviewed and approved by the sponsor, researcher, and staff on the basis of scientific merit and absence of competing interests. After the proposal has been approved, data can only be shared through a secure online platform after a data access and a confidentiality agreement are signed.

Declaration of interests

MCB, BMC, GJV, NNF, PMMG, RH, BALF, and RTC received research funding from Butantan Institute during the conduct of this study. RP, MTRPC, APB, JPS, and ROP were employees of Butantan Institute during the conduct of this study. HAB, EGP, GGP, SCSV and DC are employees of Butantan Institute. All other authors declare no competing interests

Acknowledgments

This study was only possible thanks to the extraordinary commitment of people linked to several organizations. Instituto Butantan staff left their families and worked full-time in Serrana during the immunization period to monitor, coordinate and support the vaccination centers (Andressa Freitas da Silva, Andressa Gabriela Moura de Oliveira, Arlete Sandra de Jesus, Auricélia Quirino Sousa, Ayla Sol Agassi Guimarães, Bruna Colli Moreira, Elis Regina Alves Pinto, Fábia Luzia Cestari, Gabrielle Maria Cordeiro Mongs, Juliana Araujo, Juliana Cassano Carvalhal, Juliana Souza Nakandakare, Lucimar Pereira de Souza, Patricia Teraza Bastos Pereira, Quetura Oliveira Silva, Renan Moreira dos Santos, Renata Carina dos Santos Giacon, Sharon Guedes Ferreira, Vanessa Evelin Jesus Vilches Sant´anna). Such dedication was vital for the study's success. The authors are in debt to Juliana Araújo and her team in Human Resources, Romulo Xavier de Souza and his Central Warehouse team and Vivian Retz and her Communication team at Butantan for their active involvement in the study since the very beginning. Other Instituto Butantan departments provided invaluable support to the study, and we are very grateful to the Administrative, Logistics, Laboratory, and Computer Service Centre staff (Julio Cesar Ciqueira Catani, Cláudia Anania Santos da Silva, Gabriela Mauric Frossard Ribeiro, Kilmary Lincolins de Oliveira Sequeira, Richard Rodrigues dos Santos, Gabriela Ribeiro, Alex Ranieri Jerônimo Lima). The Serrana State Hospital was the foundation for this study and we thank all the staff, especially Eduardo Lopes Seixas, Anderson Mendonça Jabur, Cassia Fernanda Sales de Lima Dias, Bruno Belmonte Marinelli

Gomes, Manuela de Paula Pereira and Maria Aline Sprioli. The work from CDHU and Diagonal staff was the basis of our in-depth knowledge of the Serrana territory (Ricardo de Almeida Nobre, Maristela Valenciano Achilles, and Shirley Andreatta). We want to emphasize and recognize the availability to provide help from the Municipal and State authorities, particularly in the Health and Education areas. We want to underscore the engagement of Serrana mayors, Leonardo Caressato Capitelli and Valerio Galante, and Health Secretaries, Leila Gusmão and José Carlos Moura, and the local officers. Moreover, the commitment of community and religious leaders to promote the study in the population was outstanding. Finally, we want to praise the people of Serrana who embraced the study and joined with the research team to resolve the scientific aims of Projeto S.

References

1. Lurie N, Keusch GT, Dzau VJ. Urgent lessons from COVID 19: why the world needs a standing, coordinated system and sustainable financing for global research and development. *Lancet* 2021; **397**(10280): 1229-36.
2. Berlin DA, Gulick RM, Martinez FJ. Severe Covid-19. *N Engl J Med* 2020; **383**(25): 2451-60.
3. Gandhi RT, Lynch JB, Del Rio C. Mild or Moderate Covid-19. *N Engl J Med* 2020.
4. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 2020; **383**(27): 2603-15.
5. Palacios R, Patiño EG, de Oliveira Piorelli R, et al. Double-Blind, Randomized, Placebo-Controlled Phase III Clinical Trial to Evaluate the Efficacy and Safety of treating Healthcare Professionals with the Adsorbed COVID-19 (Inactivated) Vaccine Manufactured by Sinovac - PROFISCOV: A structured summary of a study protocol for a randomised controlled trial. *Trials* 2020; **21**(1): 853.
6. Tanriover MD, Doğanay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *Lancet* 2021; **398**(10296): 213-22.
7. Russell RL, Pelka P, Mark BL. Frontrunners in the race to develop a SARS-CoV-2 vaccine. *Can J Microbiol* 2021; **67**(3): 189-212.

8. Hodgson SH, Mansatta K, Mallett G, Harris V, Emary KRW, Pollard AJ. What defines an efficacious COVID-19 vaccine? A review of the challenges assessing the clinical efficacy of vaccines against SARS-CoV-2. *Lancet Infect Dis* 2021; **21**(2): e26-e35.

9. The Gambia Hepatitis Intervention Study. The Gambia Hepatitis Study Group. *Cancer Res* 1987; **47**(21): 5782-7.

10. Bellan SE, Pulliam JR, Pearson CA, et al. Statistical power and validity of Ebola vaccine trials in Sierra Leone: a simulation study of trial design and analysis. *Lancet Infect Dis* 2015; **15**(6): 703-10.

11. Doussau A, Grady C. Deciphering assumptions about stepped wedge designs: the case of Ebola vaccine research. *J Med Ethics* 2016; **42**(12): 797-804.

12. Hemming K, Haines TP, Chilton PJ, Girling AJ, Lilford RJ. The stepped wedge cluster randomised trial: rationale, design, analysis, and reporting. *BMJ* 2015; **350**: h391.

13. Mascarenhas J, Kumar PDP. Participatory mapping and modelling users' notes. *PLA Notes* 1991; (12).

14. Jara A, Undurraga EA, Gonzalez C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *N Engl J Med* 2021; **385**(10): 875-84.

15. Hitchings MDT, Ranzani OT, Torres MSS, et al. Effectiveness of CoronaVac among healthcare workers in the setting of high SARS-CoV-2 Gamma variant transmission in Manaus, Brazil: A test-negative case-control study. *Lancet Reg Health Am* 2021; **1**: 100025.

16. Vasileiou E, Simpson CR, Shi T, et al. Interim findings from first-dose mass COVID-19 vaccination roll-out and COVID-19 hospital admissions in Scotland: a national prospective cohort study. *Lancet* 2021; **397**(10285): 1646-57.

17. Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *N Engl J Med* 2021; **384**(15): 1412-23.

18. Thompson MG, Burgess JL, Naleway AL, et al. Interim Estimates of Vaccine Effectiveness of BNT162b2 and mRNA-1273 COVID-19 Vaccines in Preventing SARS-CoV-2 Infection Among Health Care Personnel, First Responders, and Other Essential and Frontline Workers - Eight U.S. Locations, December 2020-March 2021. *MMWR Morb Mortal Wkly Rep* 2021; **70**(13): 495-500.

19. Eichner M, Schwehm M, Eichner L, Gerlier L. Direct and indirect effects of influenza vaccination. *BMC Infect Dis* 2017; **17**(1): 308.

20. Mostaghimi D, Valdez CN, Larson HT, Kalinich CC, Iwasaki A. Prevention of host-to-host transmission by SARS-CoV-2 vaccines. *Lancet Infect Dis* 2021.

21. Palacios R. Forgotten, but not forgiven: facing immunization challenges in the 21 (st) century. *Colomb Med (Cali)* 2018; **49**(3): 189-92.

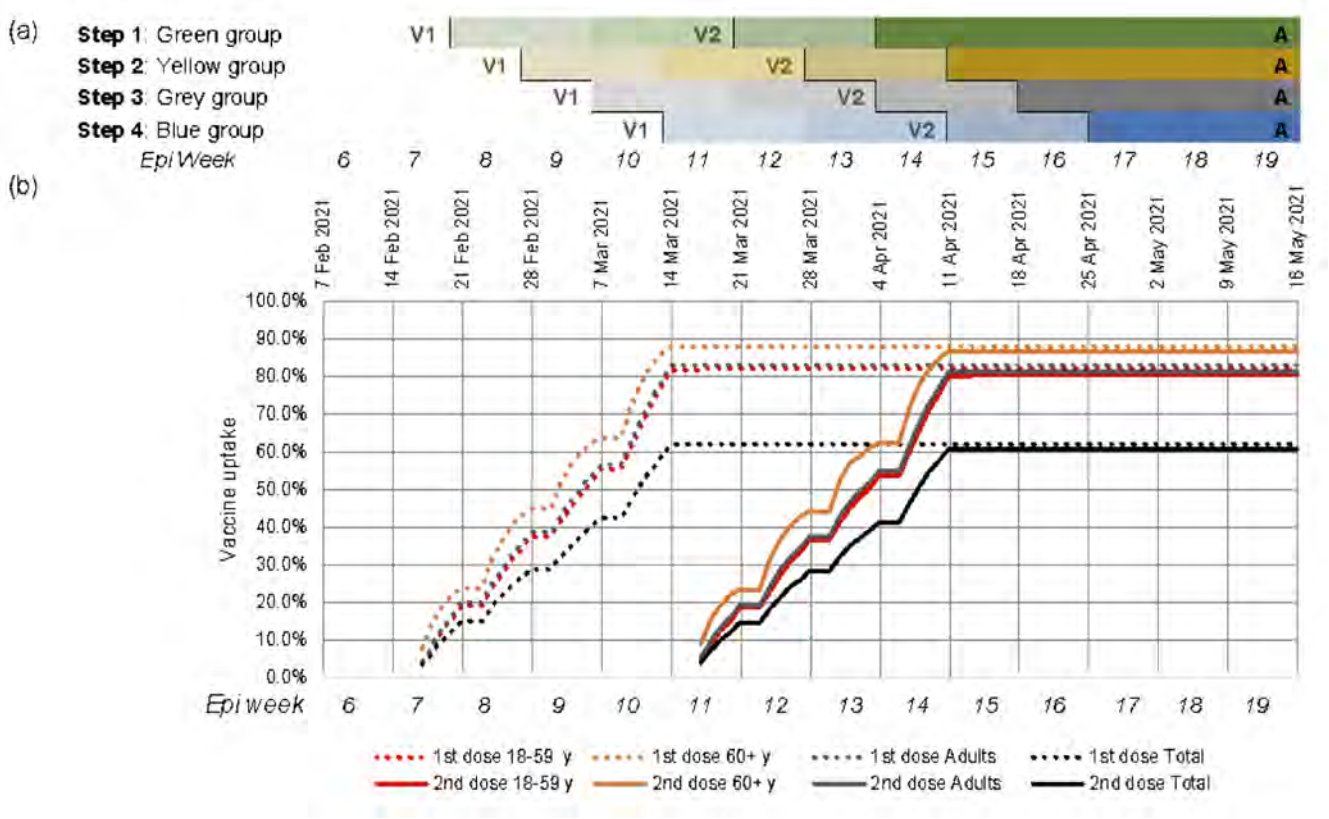
22. Gramacho WG, Turgeon M. When politics collides with public health: COVID-19 vaccine country of origin and vaccination acceptance in Brazil. *Vaccine* 2021; **39**(19): 2608-12.

Figure legends

Figure 1. Study design and vaccine uptake in the population of Serrana, Brazil, 2021. The panel (a) shows the study periods and time of intervention for each step/group. The Control Period is shown in white. The Transition Period is shown with a diagonal pattern. The Intervention Period is in solid colors. V1: 1st dose of vaccine. V2: 2nd dose of vaccine. A: is the cut-off for analysis. The panel (b) shows the vaccine uptake per dose and age group and overall population.

Figure 2. Vaccina coverage and incidence rate ratios for the entire population (a) and for each color-coded groups (b-e) for symptomatic Covid-19 cases, Serrana, Brazil, 2021.

Figure 3. Cumulative incidence for Covid-19-related hospitalization and death between epidemiological weeks 6 and 19 in Serrana and other cities in the region with over 30,000 inhabitants, 2021.


(a)
Step 1: Green group
Step 2: Yellow group
Step 3: Grey group
Step 4: Blue group
V1
V2
A
Epi Week 6 7 8 9 10 11 12 13 14 15 16 17 18 19
(b)
7 Feb 2021
14 Feb 2021
21 Feb 2021
28 Feb 2021
7 Mar 2021
14 Mar 2021
21 Mar 2021
28 Mar 2021
4 Apr 2021
11 Apr 2021
18 Apr 2021
25 Apr 2021
2 May 2021
9 May 2021
16 May 2021
Vaccine uptake
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Epi week 6 7 8 9 10 11 12 13 14 15 16 17 18 19
1st dose 18-59 y
2nd dose 18-59 y
1st dose 60+ y
2nd dose 60+ y
1st dose Adults
2nd dose Adults
1st dose Total
2nd dose Total

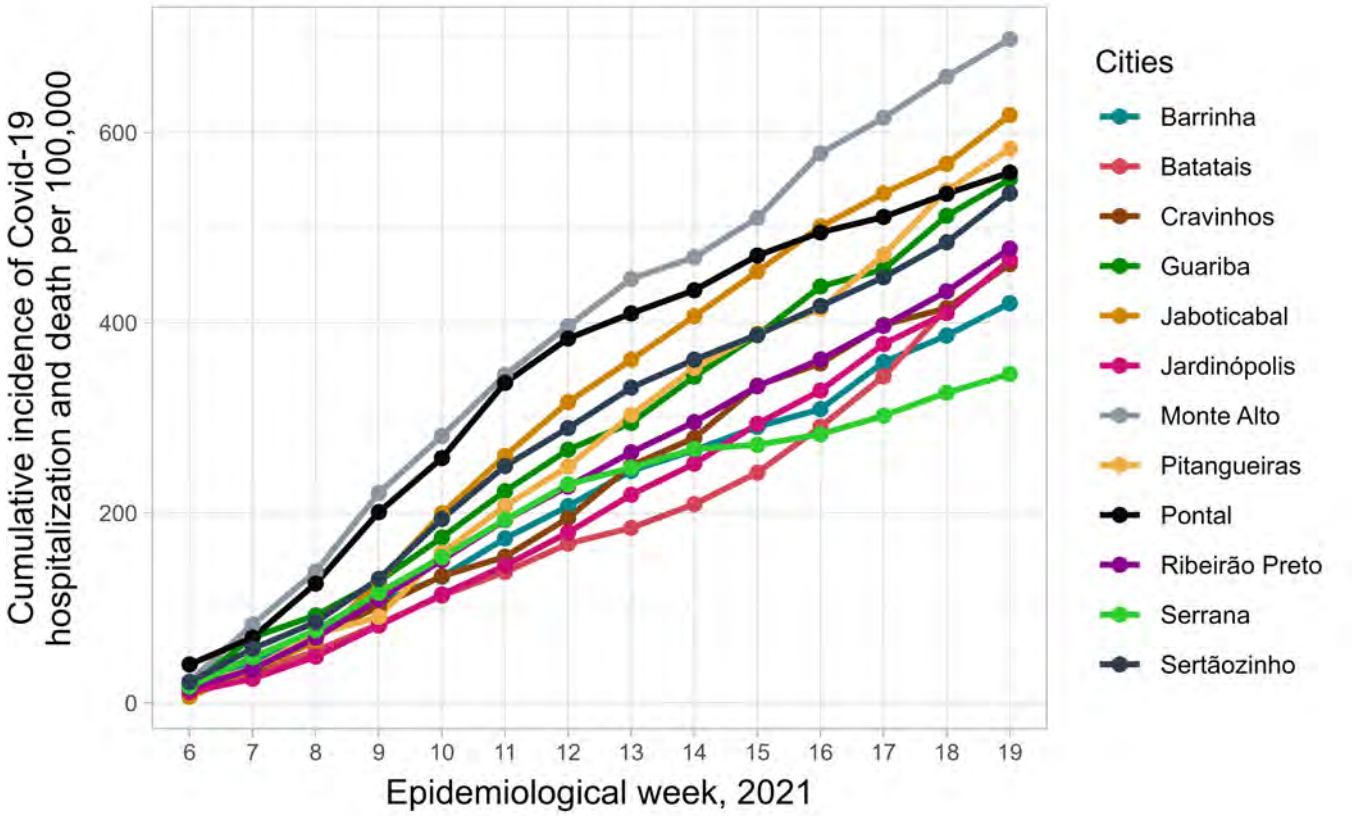

Cumulative incidence of Covid-19 hospitalization and death per 100,000
0
200
400
600
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Epidemiological week, 2021
Cities
Barrinha
Batatais
Cravinhos
Guariba
Jaboticabal
Jardinópolis
Monte Alto
Pitangueiras
Pontal
Ribeirão Preto
Serrana
Sertãozinho

Table 1. Characteristics of the Study Population, Overall, per Group, and According to Vaccination Status, Serrana, Brazil, 2021.

Characteristics	Overall	Green Group	Yellow Group	Grey Group	Blue Group
Estimated population					
Total Urban Population (n, %)	44,183 (100)	10,716 (24·3)	10,399 (23·5)	9,918 (22·4)	13,150 (29·8)
Total Adults (n, %)	33,074 (74·9)	8,026 (74·9)	7,835 (75·3)	7,323 (73·8)	9,890 (75·2)
0-17yr (n, %)	11,109 (25·1)	2,690 (25·1)	2,564 (24·7)	2,595 (26·2)	3,260 (24·8)
18-59yr (n, %)	28,104 (63·6)	6,704 (62·6)	6,586 (63·3)	6,319 (63·7)	8,495 (64·6)
≥60yr	4,970 (11·2)	1,322 (12·3)	1,249 (12·0)	1,004 (10·1)	1,395 (10·6)
Vaccinated with at least one dose					
Total Urban Population (n, %)	27,406 (62·0)	6,764 (63·1)	6,203 (59·6)	6,026 (60·8)	8,413 (64·0)
Total Adults (n, %)	27,406 (82·9)	6,764 (84·3)	6,203 (79·2)	6,026 (82·3)	8,413 (85·1)
18-59yr (n, %)	23,041 (82·0)	5,549 (82·8)	5,166 (78·4)	5,091 (80·6)	7,235 (85·2)
≥60yr	4,365 (87·8)	1,215 (91·9)	1,037 (83·0)	935 (93·1)	1,178 (84·4)
Fully vaccinated					
Total Urban Population (n, %)	26,891 (60·9)	6,647 (62·0)	6,084 (58·5)	5,897 (59·5)	8,263 (62·8)
Total Adults (n, %)	26,891 (81·3)	6,647 (82·8)	6,084 (77·7)	5,897 (80·5)	8,263 (83·5)
18-59yr (n, %)	22,580 (80·3)	5,447 (81·3)	5,057 (76·8)	4,976 (78·7)	7,100 (83·6)
≥60yr	4,311 (86·7)	1,200 (90·8)	1,027 (82·2)	921 (91·7)	1,163 (83·4)
Gender					
Female (n, %)	13,541 (50·4)	3,344 (50·3)	3,122 (51·3)	2,959 (50·2)	4,116 (49·8)
Baseline seroconversion					
RBD-reactive IgG (n, %)	6,605 (24·6)	1,398 (21·0)	1,427 (23·5)	1,647 (27·9)	2,133 (25·8)
Serology IGT (Reactive) (n, %)	6,345 (23·6)	1,341 (20·2)	1,374 (22·6)	1,578 (26·8)	2,052 (24·8)
Comorbidities					
Diabetes (n, %)	2,172 (8·2)	574 (8·7)	522 (8·7)	494 (8·5)	582 (7·2)
Dyslipidemia (n, %)	1,352 (5·1)	337 (5·1)	338 (5·6)	268 (4·7)	409 (5·0)
Cardiovascular diseases (n, %)	260 (1·0)	74 (1·1)	67 (1·1)	46 (0·8)	73 (0·9)
Hypertension (n, %)	5,449 (20·5)	1,449 (22·1)	1,314 (21·8)	1,141 (19·7)	1,545 (18·9)
Failure to complete vaccination (n, %)	515 (1·9)	117 (1·7)	119 (1·9)	129 (2·1)	150 (1·8)

Table 2. Effectiveness of CoronaVac vaccine in preventing Covid-19 outcomes in Serrana, Brazil, 2021.

	Effectiveness	95% CI
Overall effectiveness*		
Symptomatic cases	48·1	39·2 - 55·7
Hospitalization and Death	48·1	13·2 - 69·0
Direct effectiveness**		
Symptomatic cases	80·5	75·1 - 84·7
Hospitalization and Death	95·0	86·9 - 98·1
Death	94·9	76·4 - 98·9
18-59yr direct effectiveness**		
Symptomatic cases	79·3	73·2 - 84·1
Hospitalization and Death	94·4	80·2 - 98·4
Death	93·9	45·3 - 99·3
≥60yr direct effectiveness**		
Symptomatic cases	86·4	74·5 - 93
Hospitalization and Death	96·9	86·1 -99·3
Death	96·9	73·9 - 99·6

* Overall effectiveness was estimated by comparing the case incidence in the control and intervention periods for the entire urban population.

** Direct vaccine effectiveness was calculated by comparing case incidence between fully vaccinated vs. unvaccinated participants during the intervention period.

Control period, before vaccination; Intervention period, starting six weeks after initial dose (when participants are expected have two weeks or more after full vaccination scheme) to epidemiological week 19.

1.8. CoronaVac gera alta resposta de anticorpos em profissionais de saúde com e sem infecção anterior por Covid-19, apontam estudos da Turquia

Duas pesquisas conduzidas na Turquia mostraram que a CoronaVac, vacina do Butantan e da Sinovac, produz imunidade humoral eficaz em profissionais de saúde com e sem histórico de Covid-19, com taxas de soroconversão acima de 99%. Nos indivíduos que já tiveram a infecção, o nível de anticorpos produzidos foi 1,3 vez maior do que naqueles que nunca foram infectados.

O primeiro estudo, publicado em julho de 2021, analisou 730 profissionais de saúde: 103 (14%) tinham sido previamente infectados pelo SARS-CoV-2, de forma leve ou assintomática, e 627 (83%) não tinham sido infectados. Todos os indivíduos foram imunizados com duas doses de CoronaVac em um intervalo de 28 dias.

Um mês após a segunda dose, anticorpos IgG específicos para a proteína Spike foram detectados em ambos os grupos – estudos paralelos de fase 1 e 2 mostraram soroconversão em 98% dos profissionais de saúde.

Nas pessoas previamente infectadas, os níveis de anticorpos foram significativamente maiores (média de 1220 UA/mL) do que no segundo grupo (média de 913 UA/mL). Além disso, não houve diferença nas reações adversas relacionadas à vacina entre indivíduos previamente infectados e não infectados, tanto na primeira quanto na segunda dose.

Já o segundo estudo, publicado em novembro de 2021, foi feito com 330 profissionais de saúde do Hospital da Faculdade de Medicina da Universidade de Istambul-Cerrahpaşa, com idades entre 19 e 65 anos, que foram imunizados com a CoronaVac. Destes, 255 nunca tiveram a doença e 75 tinham história prévia de Covid-19 (cinco casos assintomáticos, 36 leves, 31 moderados e três graves).

Amostras coletadas 28 dias após a segunda dose mostraram soroconversão de anticorpos IgG em 100% dos previamente infectados e 99,2% dos não infectados. Em todos os participantes do estudo, a taxa de eficácia da CoronaVac foi de 99,4%. No grupo sem infecção, o título médio de anticorpos foi de 48,4 UA/mL após a primeira dose do imunizante, que aumentou para 707,1 UA/mL depois da segunda dose. Já entre os profissionais com história prévia de Covid-19, a média de anticorpos era de 301,9 UA/mL antes da vacinação, elevando para 1331,2 UA/mL depois da primeira dose e se mantendo em níveis semelhantes após a segunda.

Em suma, os participantes que já tiveram Covid-19 desenvolveram taxa de soroconversão significativamente maior após a primeira dose da vacina do que os participantes sem histórico da doença, porém as taxas de desenvolvimento de anticorpos após a imunização completa foram semelhantes, entre 99% e 100%.

Publicados em: 22/11/2021 e 29/07/2021

Artigo

Vaccine 40 (2022) 52–58

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Inactive SARS-CoV-2 vaccine generates high antibody responses in healthcare workers with and without prior infection

Harika Oyku Dinc [a,b], Nese Saltoglu [c,*], Gunay Can [d], Ilker Inanc Balkan [c], Beyhan Budak [c], Dogukan Ozbey [b], Bilge Caglar [c], Rıdvan Karaali [c], Bilgul Mete [c], Yesim Tuyji Tok [b], Yagmur Ersoy [b], Mert Ahmet Kuskucu [b], Kenan Midilli [b], Sevgi Ergin [b], Bekir Sami Kocazeybek [b]

[a] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Bezmialem Vakif University Istanbul, Turkey
[b] Department of Medical Microbiology, Cerrahpaşa Medical Faculty, Istanbul University-Cerrahpaşa, Istanbul 34098, Turkey
[c] Department of Infectious Diseases and Clinical Microbiology, Cerrahpaşa Medical Faculty, Istanbul University-Cerrahpaşa, Istanbul 34098, Turkey
[d] Department of Public Health, Cerrahpaşa Medical Faculty, Istanbul University-Cerrahpaşa, Istanbul 34098, Turkey

ARTICLE INFO

Article history:
Received 8 June 2021
Received in revised form 9 October 2021
Accepted 17 November 2021
Available online 22 November 2021

Keywords:
COVID-19
CoronaVac
Chemiluminescent microparticle immunoassay
Healthcare workers
the anti-spike/RBD antibody

ABSTRACT

Background and Objectives: Healthcare workers (HCWs) were among the first groups to be vaccinated in Turkey. The data to be obtained by the vaccination of HCWs would guide wide spread vaccination programs.

Materials and Methods: The study included 330 HCWs working at Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty Hospital and vaccinated with inactive CoronaVac (Sinovac Life Sciences, China) SARS-CoV-2 vaccine in two doses (28 days apart). Anti-Spike /RBD IgG levels were measured 14 days after the first dose and 28 days after the second dose. Chemiluminescent microparticle immunoassay (CMIA) (ARCHITECT IgG II Quant test, Abbott, USA), which is 100% compatible with plaque reduction neutralization test (PRNT), was used.

Results: Of the participants, 211 (63.9%) were female, 119 (36.1%) were male, and mean age was 39.6 ± 7.7 years. In those without prior COVID-19 history; (n = 255) antibody positivity was detected as 48.2% (95% CI: 42.1–54.3) 14 days after the first dose of vaccine, and 99.2% (95% CI: 98.1–100) at day 28 after the second dose. Antibody titers were significantly lower in patients with hypertension (p = 0.011). In those with prior history of COVID-19 (n = 75); both the antibody positivity rates after the first vaccine (48.2% vs 100%, p = 0.000) and the anti-spike/RBD antibody levels after the second vaccine (with a ≥ 1050 AU/mL titer equivalent to PRNT 1/80 dilution) was significant than infection-naive group (25.9% vs. 54.7%, p = 0.000). Antibody positivity after two doses of vaccination for all study group was 99.4% (95% CI: 98.6–100).

Conclusions: Two doses CoronaVac produce effective humoral immunity in HCWs. Antibody response is significantly higher in those with prior history of COVID-19 than infection-naive group. Given no significant benefit of the second dose, a single shot of vaccination may be sufficient for those with prior history of COVID-19. Monitoring humoral and cellular immune responses, considering new variants, is required to validate this approach.

1. Introduction

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to cause high morbidity and mortality worldwide [1]. As of Oct 4, 2021 worldwide, 234.809.103 confirmed cases of SARS-CoV-2 infection had been reported, 4.800.375 of which resulted in death [2]. A total of 7.238.267 people have been infected in Turkey throughout this period, and 64.661 of these have died [3]. Despite these devastating consequences of the Covid-19 pandemic, it is promising that many vaccines are available today.

CoronaVac vaccine, produced by Sinovac Life Sciences (Beijing, China) using the conventional inactivation technique, develops immune response against the entire viral proteins including matrix, envelope, nucleoprotein structures and spike protein of

* Corresponding author at: Department of Infectious Diseases and Clinical Microbiology, Cerrahpaşa Medical Faculty, Istanbul University-Cerrahpaşa, Istanbul 34098, Turkey.
E-mail address: saltoglu@iuc.edu.tr (N. Saltoglu).

https://doi.org/10.1016/j.vaccine.2021.11.051
0264-410X/

SARS-CoV-2. In phase 2 clinical trial, 97% seroconversion was reported 28 days after CoronaVac (3 μg on day 0 and day 28) administration [4]. In the phase 3 study, efficacy rates remained high, though varying between 51 and 84%, according to the countries [5]. However, the protective efficacy of current vaccines against infection and re-infection and the duration of protection in real life, are still unclear.

In Turkey, the Ministry of Health approved the use of CoronaVac (Sinovac) on 13.01.2021, and vaccination was launched first in the healthcare workers (HCWs). At Cerrahpaşa "COVID-19 Adult Vaccination Center", the first dose of vaccines were administered to 2426 HCWs between January 15 and 25, 2021. The second vaccinations were administered in the following month.

The primary aim of this study is to quantitatively detect IgG antibody levels in blood samples of HCWs, obtained 14 days after the first dose of the vaccine and 28 days after the second dose, and to monitor the time-dependent changes in the antibody levels. HCWs who were administered SARS-CoV-2 inactivated vaccine were divided into two groups as those with prior history of COVID-19 (recovered at least 4 months ago) and those with no evidence of prior infection. The aim here is to determine whether there is a difference between antibody levels in those who have had the disease and those who have not. We also aimed to determine whether there is a difference in antibody levels between those who have had and those who have not comorbidities. The second aim of this study was to reassess antibody levels in the long term (3rd and 6th months) and to determine whether HCWs were infected with SARS-CoV-2 during this time period as an indicator of long-term protection.

2. Methods

The study included 346 healthcare professionals who were administered the first dose of CoronaVac (Sinovac Life Sciences, Beijing, China) between 15.01.2021 and 28.01.2021, and the second dose between 18.02.2021 and 05.03.2021. The study population consisted of those who had the first dose of the vaccine between 15 and 25 January 2021. By evaluating the literature data, the sample size was determined to be at least 310 individuals within the 95% confidence interval, when the 75% margin of error of the expected antibody positivity after the second dose was taken into consideration and the 5% design effect as 1.2. The number of samples was increased by 10% due to dropout problems that may be encountered in the follow-up. It was planned to collect peripheral blood samples from the participants 14 days after the first dose and 28 days after the second dose to investigate the presence of SARS-CoV-2 IgG. At various stages of the study, 2 healthcare workers who had COVID-19 and 14 who had not had COVID-19 voluntarily left the study (Fig. 1).

The demographic data of all participants were recorded in the follow-up form (age, gender, blood group type, the symptoms, the presence of comorbidities, etc.). Individuals with prior history of COVID-19 and native for Covid 19 had no respiratory symptoms until 14 days before the study. The antibody responses of 255 healthcare workers with COVID-19 infection-naive group and 75 healthcare workers with prior history of COVID-19 (with clinical symptoms and PCR-confirmed SARS-CoV-2 infection) at least four months ago before the study were evaluated. We also had the pre-vaccine serum samples taken for routine/study purposes from participants with prior history of COVID-19. In addition, the history of infection (diagnosis, clinical presentation, symptoms, etc.) in those who had COVID-19 and also vaccinated was evaluated together with the obtained antibody results. informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed. This study was approved by the Republic of Turkey Ministry of Health General Directorate of Health Services Scientific Research Studies Commission (Date: 26.01.2020), Istanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Scientific Research and Evaluation Commission (Date: 19.02.2021 and Number: 35131) and Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty Clinical Research Ethics Committee approval (Date: 03.02.2020 and Decision No: 23461).

In this study, the SARS-CoV-2 IgG test (ARCHITECT IgG II Quant test, Abbott, USA), which can quantitatively detect immunoglobulin G(IgG) antibodies, including neutralizing antibodies against the receptor-binding region (RBD) of the spike protein S1 subunit of SARS-CoV-2 was used by the chemiluminescent microparticle immunoassay (CMIA) method. The antibody results of studied sera were evaluated as Arbitrary Unit/mL (AU/mL). The antibody concentrations obtained in AU/mL were multiplied by the correlation coefficient of 0.142 and converted to the "Binding Antibody Unit (BAU/mL)" in the WHO's International Standard for Anti-SARS-CoV-2 immunoglobulin [6]. Accordingly, 50 AU/mL or 7.1 BAU/mL and above concentrations were considered positive. It was also reported that this test was 100% compatible with the plaque reduction neutralization test (PRNT), and a concentration of 1050 AU/mL was associated with a 1:80 dilution of PRNT [7].

The SARS-CoV-2 IgG test (ARCHITECT IgG test, Abbott, USA), which semi-quantitatively detects IgG antibodies against the Nucleocapsid protein (NCP) of SARS-CoV-2, was used in serum samples taken after both doses of healthcare workers without history of COVID-19. In the previous study conducted in our center for the diagnostic performance of antibody tests, the mean NCP IgG (2.03 S/Co) in the acute period of patients with covid 19 was evaluated as cut-off [8]. The volunteers with a concentration above 2.03 S/Co were considered to be in contact with SARS-CoV-2 and concentrations between 1.4 and 2.03 S/Co were evaluated as vaccine-induced.

2.1. Statistical analysis

The IBM SPSS statistic 21 package program was used to evaluate the data. Qualitative data are presented as number and percentage, and quantitative data are presented as median and IQR25-75. Chi-

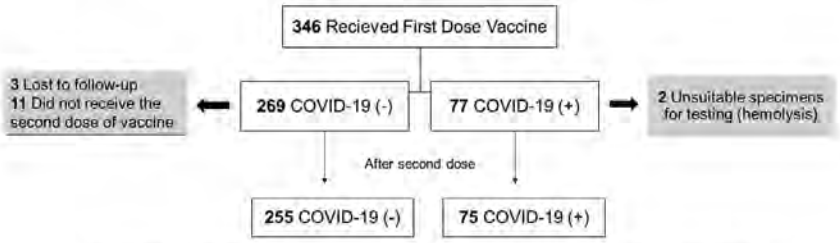


Fig. 1. Flowchart of volunteers participating in the Inactive SARS-CoV-2 Vaccine Efficacy Study.

square and Fisher's exact test were used in the evaluation of qualitative data, Student's *t* test, Mann Whitney *U* test and Kruskal Wallis test were used in the comparison of quantitative data. Spearman analysis was used for the correlation analysis. and $p < 0.05$ value was considered significant in all analysis.

This work was supported by IU- Cerrahpaşa Scientific Research Projects Unit (Project ID: 35691).

3. Results

The ages of 330 HCWs included in this study are ranged between 19 and 65, with a mean age of 39.6 ± 7.7 years. 211 (63.9%) of the participants were female, and 119 (36.1%) were male. Of the 75 participants with prior history of COVID-19, 38 (50.7%) were male, and 37 (49.3%) were female, with a mean age of 39.53 ± 11.54 years. Of the infection-naive group, 81 (31.8%) were men, 174 (68.2%) were women, and the mean age was 39.5 2 ± 11.06 years.

Of the individuals with a prior history of COVID-19, 5 had asymptomatic COVID-19, 36 had mild, 31 had moderate, and 3 had severe clinical forms of the disease [9]. Fever(53,3%), fatigue (74,6%), arthralgia(57,3%), loss of taste and smell (69,3%) and headache(49,3%) were observed as the most common symptoms in these individuals. Of the 75 participants with a prior history of COVID-19, three had no detectable antibodies in the serum sample obtained before vaccination. The percentage of positive antibodies against the SARS-CoV-2 was 96.0% (95% CI: 91.6–100) in above group. Antibody levels were detected in all cases after the first and second doses of the vaccine. When the antibody response after two doses of vaccination was compared to the severity of COVID-19 in the group with a prior history of COVID-19, no significant difference was found ($p > 0.05$).

In the infection-naive group, the percentage of positive antibodies 14 days after the first dose of vaccine was 48.2% (95% CI: 42.1–54.3). The positive antibody percentage 28 days after the second dose of vaccine was 99.2% (95% CI: 98.1–100), and only two HCWs among this group were negative for antibody against SARS-CoV-2 (Table 1). In the total study group, the antibody positivity for SARS-CoV-2 was 99.4% (95% CI: 98.6–100) after two doses of vaccination

IgG antibody titers of over 1050 AU/mL (which is equivalent to 1:80 dilution in the plaque reduction neutralization test) were detected in 25.9% of the infection-naive group and in 54.7% of those with a prior history of COVID-19, the difference was statistically significant ($p<0.001$) (Table 1). The percentage of antibody positivity was found to be 51.1% and 42.0% in males and females after the first dose vaccination, respectively. On the other hand, the percentage of antibody positivity was found to be 99.5% and 99.2% in males and females after the second dose of vaccination, respectively. The efficacy rate of the CoronaVac vaccine was found as 99.4% in all participants, both under 40 and over 41 years old. No significant difference was detected between antibody responses according to blood groups.

Median antibody titer was 48,4 AU/mL after the first dose of vaccine in the infection-naive group, which increased to 707,1 AU/mL after the second dose, the difference was statistically significant ($p<0.001$). While the median antibody titer was 301.9 AU/mL before vaccination in participants with prior history of COVID-19, it was found to be 1331.2 AU/mL after the first dose of vaccination ($p<0.001$). After the second dose in the above group, the median antibody titer was found as 1090,0 AU/mL (Table.2) (Fig. 2) ($p>0.05$). Median antibody titers in groups with and without a prior history of COVID-19 did not differ significantly in terms of age and gender. There was a very low significant negative correlation between the age and antibody titers after the second dose in

Table 1
Evaluation of demographic data and antibody results of participants as a percentage.

	Infection-naive Group n = 255 (%)	Prior History of COVID-19 n = 75 (%)	p
Gender			
Male	81 (31,8)	38 (50,7)	,003*
Female	174 (68,2)	37 (49,3)	
Age			
<40	128 (50,2)	39 (52,0)	,784
>40	127 (49,8)	36 (48,0)	
Body-Mass Index			
Normal	120 (49,0)	34 (45,9)	,320
Overweight	89 (36,3)	33 (44,6)	
Obese	36 (14,7)	7 (9,5)	
Department			
Basic Medical Sciences	9 (4,0)	7 (9,7)	,063
Internal Medical Sciences	93 (41,3)	22 (30,6)	
Surgical Medical Sciences	59 (26,2)	26 (36,1)	
Other Staff	64 (28,4)	17 (23,6)	
Comorbidity			
Allergy	22 (8,6)	5 (6,7)	,586
Auto-immune Diseases	4 (1,6)	1 (1,3)	1,000
Neurological Disorders	2 (0,8)	2 (2,7)	,223
Malignity	2 (0,8)	0 (0,0)	,442
Diabetes Mellitus	9 (3,5)	3 (4,0)	,848
Hypertension	15 (5,9)	3 (4,0)	,773
Hypothyroidism	15 (5,9)	4 (5,3)	,858
Cronic Heart Diseases	2 (0,8)	2 (2,7)	,190
Asthma	7 (2,7)	0 (0,0)	,357
Blood Groups			
0+	69 (32,1)	17 (25,4)	,815
0-	6 (2,8)	3 (4,5)	
A+	86 (40,0)	27 (40,3)	
A-	8 (3,7)	5 (7,5)	
B+	23 (10,7)	8 (11,9)	
B-	4 (1,9)	1 (1,5)	
AB+	18 (8,4)	5 (7,5)	
AB-	1 (0,5)	1 (1,5)	
Anti-SARS-CoV-2 IgG After first dose (AU/mL)			
Negative (<50 AU/mL)	132 (51,8)	0 (0,0)	,000
Positive (>50 AU/mL)	123 (48,2)	75 (100,0)	
Anti-SARS-CoV-2 IgG After second dose (AU/mL)			
Negative (<50 AU/mL)	2 (0,8)	0 (0,0)	–
Positive (>50 AU/mL)	253 (99,2)	75 (100,0)	
Anti-SARS-CoV-2 IgG After second dose (AU/mL)			
<1050 AU/mL	189 (74,1)	34 (45,3)	,000
>1050 AU/mL	66 (25,9)	41 (54,7)	

Table 2
SARS-CoV-2 IgG averages in blood samples taken at different times from healthcare workers who have prior history of COVID-19 and who are infection naïve.

Anti-SARS-CoV-2 IgG	Infection-naive Group	Prior History of COVID-19	p
	Median (IQR 25–75)	Median (IQR 25–75)	
Before Vaccination (AU/mL)	–	301,9 (124,1–854,2)	
After First Dose (AU/mL)	48,4 (17,4–109,3)	1331,2 (900,1–2573,7)	**,000*** **
After Second Dose (AU/mL)	707,1 (426,4–1083,7)	1090,0 (612,0–1864,1)	**,000*** **

AU/mL : Antibody Unit / mililiter ; IQR : Inter Quantile Range.

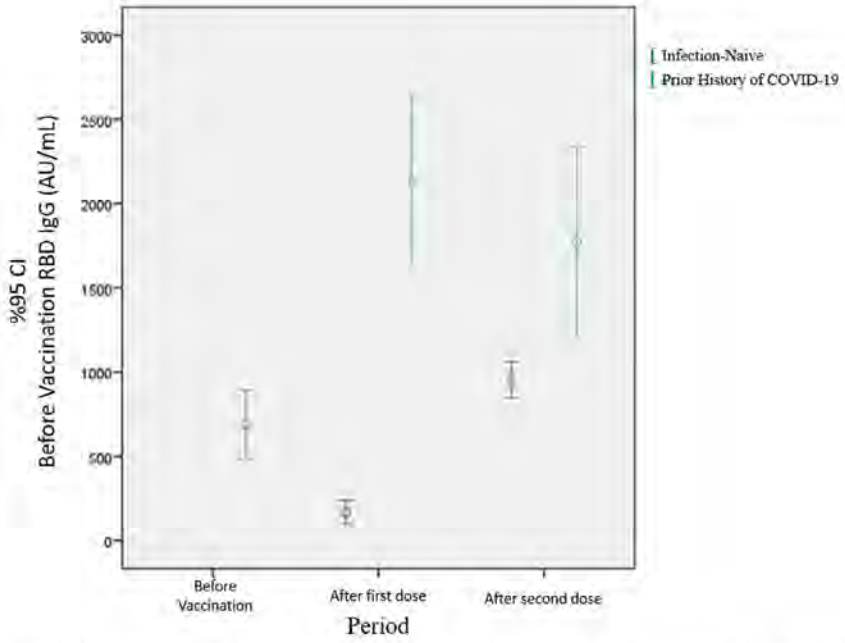


Fig. 2. SARS-CoV-2 IgG averages in blood samples taken at different times from healthcare workers who have prior history of COVID-19 and who are infection naïve.

infection-naive group (r = -0.15 p<0.05). When evaluated in terms of comorbid conditions; It was found that COVID-19 infection-naive group had significantly lower antibody titers in the presence of hypertension (p<0.05) (Table 3).

Table 3
Evaluation of antibody titers in healthcare workers according to demographic data.

	Infection-Naive Group			Prior History of COVID-19		
	n	Median (IQR)	p	n	Median (IQR)	p
Gender						
Male	81	674,4(447,3–1289,3)	,923	38	1114,6(444,5–1873,5)	,711
Female	174	720,1(420,1–1032,8)		37	1078,1(617,2–1996,9)	
Age						
<40	128	807,7(482,5–1155,9)	**,024**	39	947,5(454,8–1552,9)	,071
≥40	127	601,9(382,9–1009,4)		36	1253,2(732,8–2371,9)	
Body-Mass Index						
Normal	120	764,0(422,7–1028,8)	,546	34	806,8(444,5–1441,1)	,077
Overweight	89	626,3(388,5–1132,8)		33	1413,1(870,2–2204,4)	
Obese	36	619,0(460,4–1032,5)		7	1055,5(582,5(1269,7)	
Department						
Basic Medical Sciences	9	729,1(358,7–1632,5)	,846	7	883,6(438,6–1864,1)	,500
Internal Medical Sciences	93	703,0(427,4–1035,7)		22	974,1(470,7–2375,5)	
Surgical Medical Sciences	59	767,8(477,4–1241,9)		26	1266,8(717,0–2039,0)	
Other Staff	64	735,0(459,6–1124,6)		17	970,7(419,4–1485,4)	
Allergy						
Absent	233	705,6(424,0–1087,9)	,719	70	1056,7(562,1–1711,0)	
Present	22	842,8(466,1–1074,0)		5	3382,0(1816,4–6631,8)	
Diabetes Mellitus						
Absent	246	720,1(415,6–1105,5)	,268	72	1084,1(589,9–1858,0)	–
Present	9	488,9(464,9–674,0)		3	1152,6(738,6	
Hypertension						
Absent	240	731,5(445,4–1134,6)	**,011**	72	1068,0(589,9–1820,4)	–
Present	15	488,9(255,3–674,4)		3	2374,9(1152,6	
Hypotroidism						
Absent	240	706,4(422,8–1089,9)	,621	71	1090,0(582,5–1839,8)	–
Present	15	896,9(450,0–1042,0)		4	1948,3(708,4–3440,7)	
Comorbidity						
Absent	196	**745,2(435,8–1221,3)**	**0,041**	62	1056,6(495,4–1781,7)	0,203
Present	59	584,6(386,8–989,9)		13	1152,6(854,6–3153)	

IQR : Inter Quantile Range.

Table 4
Comparison of demographic data and post-vaccine antibody responses by viral exposure in 255 infection-naive participants.

COVID-19 naive	NCP IgG Negative(n: 231)	NCP IgG Positive(n: 24)	p
Gender; n (%)	162	12	
- *Female*	(70,1%)69	(50%)12	0,044
- *Male*	(29,9%)	(50%)	
Age; Mean (SD)	39,58 (11,152)	39,14 (10,631)	0,828
After First Dose (AU/mL); Median (IQR25-75)	46,7(15,9–96,6)	98,3(30,9–604,2)	,000***
After Second Dose (AU/mL); Median (IQR25-75)	672,7(401,2–1012,3)	1687,1(1013,5–2995,1)	,000***

NCP: Nucleocapside ; SD : Standard Deviation ; AU/mL : Antibody Unit / mililiter ; IQR : Inter Quantile Range.

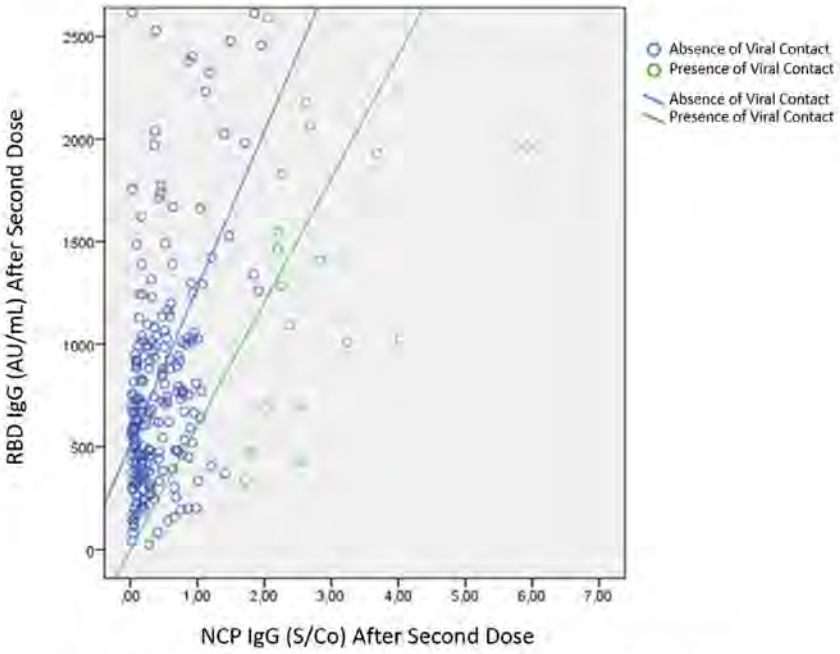


Fig. 3. SARS-CoV-II (RBD) IgG results by depending on viral contact in the Infection-Naive group.

In COVID-19 infection-naive group, NCP IgG positivity was detected in 35 participants. In this group, SARS-CoV-2 NCP IgG seropositivity due to contact with the virus was detected in a total of 24 participants (12 females, 12 males), 4 after the first dose and 20 after the second dose. These 24 participants were questioned retrospectively, and it was found that they did not have any clinical signs of COVID-19. It was observed that the SARS-CoV-2 IgG (RBD/S1) antibody titer values of these 24 individuals were 2-fold higher than the median antibody titer values of the people (n:231) who did not have contact with the virus and without a prior history of COVID-19 (Fig. 3) (Table 4). A low degree of significant positive correlation was observed between NCP IgG values and RBD/S1 IgG titers in those without viral exposure ($r = 0.41$, $p<0.001$). A moderately significant positive correlation was observed in those with viral contact ($r = 0.59$, $p<0.01$). Regarding the gender distribution among those in contact with the virus, males were found to be significantly dominant ($p<0.05$).

4. Discussion

Ensuring widespread access to a safe and effective vaccine against the pandemic has been the most vital challenge of the past year. Immediate vaccination of HCWs is a critical step both in mitigating the pandemic and in guiding widespread vaccination programs. In this study, the antibody response rates and vaccine efficacy in HCWs, both infection-naïve and with a prior history of COVID-19, with and without comorbidities were determined. Those with a prior history of COVID-19 developed significantly higher antibody responses after the first dose of vaccine (96.4% vs. 48%), yet the antibody development rates after the second dose were similar (%99 vs. %100). Hence, there was a significant decrease in the median antibody titers of HCWs with hypertension (488.9 vs. 731.5) without prior history of infection. There was no difference between the two groups when evaluated in terms of other comorbid diseases and blood groups. We also observed that the antibody response detected in two HCWs in the infection-naive group was below the protective level (<50 AU/mL). One of these HCWs was a diabetic patient over 60 years old and the other was receiving immunosuppressive therapy. No significant difference was detected in HCWs with prior COVID-19 in terms of comorbid diseases.

In addition to basic measures such as hand hygiene, social distancing, and universal use of mask; a safe and effective vaccine is pivotal in curbing the pandemic. In this context, various vaccines, based on various production methodologies are currently available worldwide with emergency use approval. The efficacy rates of AstraZeneca/Oxford, Johnson and Johnson, Moderna, Pfizer/BionTech, and Sinopharm, which are on the WHO's emergency use list,

have been reported as 63.09%, 66%, 92%, 95%, and 79%, respectively [10]. The efficacy rates of CoronaVac (Sinovac), which received WHO emergency use approval on 01.06.2021, were announced as 51% in Brazil, 65% in Indonesia and 84% in Turkey, according to Phase 3 studies [5].

Although the efficacy of COVID-19 vaccines has been investigated and different efficacy rates have been reported, the real-life efficacy data are not yet fully elucidated. In a study conducted in Israel, it was reported that the BNT162b2 (Pfizer/BionTech) vaccine had an efficacy of 66–85% in reducing SARS-CoV-2 positive cases and efficacy over 90% in reducing hospitalizations [11]. In a study with healthcare professionals in Brazil, the efficacy rate of CoronaVac, two weeks after the second dose of CoronaVac was reported as 50.7% (95% CI: 33.3–62.5%). It has also been reported that this efficacy rate was increased further in the next two weeks (68.4% at 4 weeks and 73.8% at 5 weeks) [12]. After vaccination, 142 samples that were detected PCR positive, were evaluated for SARS-CoV-2 variants and 47% (67) of these samples were found to harbour mutations related to "Variant of Concern (VOC)" announced by WHO, majority of which were P.1. variant [12]. It is crucial to monitor the efficacy of existing COVID-19 vaccines for new variants of SARS-CoV-2, including B.1.1.7, 501Y.V2 and P.1. In a study investigating the efficacy of inactivated SARS-CoV-2 vaccines in Jordanian and Egyptian populations, although it has been reported to reduce the risk of symptomatic COVID-19 risk, but its efficacy against variants has not been tested [13]. While new variants are alarming, it is promising to observe a significant reduction over time by vaccination in confirmed symptomatic COVID-19 cases [12]. We aim to continue monitoring vaccine efficacy in the participants against these emerging SARS-CoV-2 variants in the second phase of our study.

One of the most critical problems in COVID-19 vaccination is the duration and the extent of protection of the developed antibodies. Therefore, it was planned to follow up the vaccinated patients for up to 6 months. SARS-CoV-2 NCP IgG positivity was detected in 35 participants. Although it has been suggested that anti-nucleocapsid antibodies may also develop in response to inactivated SARS-CoV-2 vaccines, preclinical studies demonstrate their levels to be approximately 30 times lower than anti-RBD antibodies [14]. No data were presented regarding IgG response against the nucleocapsid of SARS-CoV-2 in the Phase1/2 study of the CoronaVac vaccine. However, B cells are known to generate antibody responses initially to the nucleocapsid antigens in individuals exposed to the SARS-CoV-2, and nucleocapsid IgG is known to serve as one of the clinical diagnostic markers [15–17]. Since we could not detect NCP IgG in 86.27% of those without a prior history of COVID-19 in this study, the possibility of contact with the virus during this process worths considering for the individuals who were NCP IgG positive. Based on the NCP IgG results, we suggest that 11 people may have developed a vaccine-induced NCP IgG response, while 24 people may have developed a virus-induced NCP IgG response. In addition, when we questioned these 24 people for 60 days from the beginning of the vaccination process, these people did not report any symptoms or clinical findings and only 12 of these people had a history of close contact with a COVID-19 positive individual. These findings suggest that people (n:24) with an elevated positive NCP IgG result may have had the COVID-19 asymptomatically and very recently, probably before the second vaccination or more earier but later than the contact time of the COVID-19 group with the COVID-19. Although the COVID-19 inactivated vaccines don't provide a 100% protection against infection, we suggest that they may effectively prevent severe disease since none of the HCWs that were followed during this period developed a symptomatic COVID-19 infection.

Determining the duration of protective efficacy and the requirement for a booster dose remain among unsolved problems. It was reported that IgG antibodies developed by the COVID-19 infection largely protects from re-infection for about 6 months in a study conducted in healthcare professionals who had COVID-19 [18]. In the SIREN study conducted on 20,787 HCWs in England, it was reported that the protection rate for the first 5 months after infection was 83%, but the contagiousness of healthcare personnel could continue during this period, and attention was drawn to the possibility of re-infection [19].

Data are scarce regarding the protective efficacy of natural antibodies developed post-infection. Therefore, vaccination is recommended regardless of prior COVID-19 infection status [20]. One of the critical questions is whether a single dose of vaccine will be sufficient for these people. Antibody positivity in the group that had the COVID-19 before vaccination was 96%. It was also observed that the antibody titers of 75 people who had COVID-19 at least four months ago increased three-fold after the first dose of vaccination. Although there is a slight decrease in the median antibody titers (16%) after the second dose, the median antibody titers are approximately 2.5 times higher than in the infection-naïve group. When all data are evaluated together, it can be suggested that a single dose of vaccine administered 3–6 months apart to the infection may be sufficient for those with confirmed prior COVID-19, thus the limited resources of vaccine can be mobilized to a larger extent of vulnerable populations. Memory B and T cell responses play a vital protective role in case of re-exposure to the virus. It is well documented that T cell response develops within the first 14 days after a single dose of the CoronaVac vaccine, while B cell response improves after the second dose [21]. Given the results of recent studies, including ours, it is still vital to administer vaccines in two doses to those with no known exposure to SARS-CoV-2.

There are very limited number of studies for the efficacy of COVID-19 vaccines in those with chronic diseases and those who have had COVID-19 before. Our study, comprising a population of HCWs with and without chronic diseases besides those with and without prior infection, provides a set of real life data. Since only the Sinovac vaccine was available in Turkey during this period, the results of this vaccine were evaluated in the healthcare personnel. The inability to evaluate the cellular immune responses of the participants is among major limitations of this study, conducted in a single center, on a limited population. Although, the possibility of exposure to the SARS-CoV-2 virus between the blood collection periods after the first and second dose vaccination was taken into account, the PCR test, which is considered the gold standard in acute diagnosis of COVID-19, could not be routinely performed on the participants before the study. Instead, nucleocapsid IgG-targeted antibody testing was used for the serum samples obtained between the indicated time periods.

Demonstrating the presence of the SARS-CoV-2-specific neutralizing antibodies developed after infection and vaccination is very important in terms of protective immunity. However, it is difficult to perform PRNT in routine practice, which is the reference standard method, due to the need for special laboratory conditions with biosafety level 3 (BSL3) and experienced specialists. Therefore, we used an antibody test with 100% correlation with PRNT and another limiting factor is that the evaluation was made according to the cut-off value of the manufacturer. Although the World Health Organization (WHO) is working to establish a standard for antibody tests with a reference serum sample (NIBSC code 20/136) and its dilutions, a safe cut-off value indicating the protective immunity has not been defined yet [22]. Only the FDA has defined a cut-off value for convalescent plasma, and this value is > 840 AU/ml for the test we used in this study [23].

As a result, while the vaccine response was 45% two weeks after the first dose in HCWs, the rate of it reached to 99% within one month after the second dose. Two doses of inactivated CoronaVac (Sinovac) vaccine produced effective humoral immunity in HCWs.

Response to the vaccine is similar following the first and second doses in those with a prior history of COVID-19. Moreover, antibody levels are significantly higher in comparison to the infection-naive group. Given no significant benefit of the second dose, in terms of antibody titers, a single shot of vaccination may be sufficient for those with prior history of COVID-19. Monitoring humoral and cellular immune responses, considering new variants, is required to validate this approach.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We thank Songül Gedik Koç, Feray Ergen, Yasemin Öztürk, Elfide Şentürk Yurdatapan, Demet Durgun Karakaş, Nafiye Güder and Fatih Hekimoğlu for thier contributions the paper.

Financial support

This work was supported by IU- Cerrahpaşa Scientific Research Projects Unit (Project ID: 35691).

References

[1] Walker PGT, Whittaker C, Watson OJ, Baguelin M, Winskill P, Hamlet A, et al. The impact of COVID-19 and strategies for mitigation and suppression in low- and middle-income countries. Science 2020;369(6502):413–22. https://doi.org/10.1126/science.abc0035.

[2] Coronavirus disease (COVID-2019) situation reports. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports [Accessed 4 Oct 2021]

[3] Republic of Turkey, Ministry of Health COVID-19 Information Platform https://covid19.saglik.gov.tr/ [Accessed 4 Oct 2021]

[4] Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021;21(2):181–92. https://doi.org/10.1016/S1473-3099(20)30843-4.

[5] WHO. Evidence Assessment: Sinovac/CoronaVac COVID-19 vaccine. https://cdn.who.int/media/docs/default-source/immunization/sage/2021/april/5_sage29apr2021_critical-evidence_sinovac.pdf [Accessed 2 June 2021]

[6] World Health Organization (2020). Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody. WHO/BS/2020.2403. Available at: https://www.who.int/publications/m/item/WHO-BS-2020.2403 [Accessed 2 June 2021]

[7] Abbott SARS-CoV-2 Immunoassays https://www.corelaboratory.abbott/int/en/offerings/segments/infectious-disease/sars-cov-2 [Accessed 2 June 2021]

[8] Dinç HÖ, Özdemir YE, Alkan S, Güngördü Dalar Z, Gareayaghi N, Sirekbasan S et.al. Evaluation of the Diagnostic Performance of Different Principles of SARS-CoV-2 Commercial Antibody Tests in COVID-19 Patients. 2021;55(2):207-222/doi: 10.5578/mb.20219907

[9] Bhimraj A, Morgan RL, Shumaker AH, Lavergne V, Baden L, Cheng VC et al. Infectious Diseases Society of America Guidelines on the Treatment and Management of Patients with COVID-19. Infectious Diseases Society of America 2021; Version 4.2.0. Available at https://www.idsociety.org/practice-guideline/covid-19-guideline-treatment-and-management/ [Accessed 2 June 2021]

[10] WHO. Strategic Advisory Group of Experts on Immunization (SAGE): COVID-19 vaccines technical documents. https://www.who.int/groups/strategic-advisory-group-of-experts-on-immunization/covid-19-materials [Accessed 2 June 2021]

[11] Aran D. Estimating real-world COVID-19 vaccine effectiveness in Israel. medRxiv preprint doi: https://doi.org/10.1101/2021.02.05.21251139

[12] Hitchings MDT, Ranzani OT, Scaramuzzini Torres MS, Barbosa de Oliveira S, Almiron M, Said R et.al. Effectiveness of CoronaVac in the setting of high SARS-CoV- 2 P.1 variant transmission in Brazil: A test-negative case-control study. MedRxiv preprint doi: https://doi.org/10.1101/2021.04.07.21255081; this version posted April 7, 2021.

[13] Al Kaabi N, Zhang Y, Xia S, Yang Y, Al Qahtani MM, Abdulrazzaq N, et al. Effect of 2 Inactivated SARS-CoV-2 Vaccines on Symptomatic COVID-19 Infection in Adults: A Randomized Clinical Trial. JAMA 2021;326(1):35. https://doi.org/10.1001/jama.2021.8565.

[14] Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science 2020;369(6499):77–81. https://doi.org/10.1126/science.abc1932.

[15] Tan Y-J, Goh P-Y, Fielding BC, Shen S, Chou C-F, Fu J-L, et al. Profiles of antibody responses against severe acute respiratory syndrome coronavirus recombinant proteins and their potential use as diagnostic markers. Clin Diagn Lab Immunol. 2004;11(2):362–71. https://doi.org/10.1128/CDLI.11.2.362-371.2004.

[16] Wu H-S, Hsieh Y-C, Su I-J, Lin T-H, Chiu S-C, Hsu Y-F, et al. Early detection of antibodies against various structural proteins of the SARS-associated coronavirus in SARS patients. J Biomed Sci. Jan-Feb 2004;11(1):117–26. https://doi.org/10.1007/BF02256554.

[17] Liu L, Liu W, Zheng Y, Jiang X, Kou G, Ding J, et al. A preliminary study on serological assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 238 admitted hospital patients. Microbes Infect. May-Jun 2020;22 (4-5):206–11. https://doi.org/10.1016/j.micinf.2020.05.008.

[18] Lumley SF, O'Donnell D, Stoesser NE, Matthews PC, Howarth A, Hatch SB, et al. Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers. N Engl J Med. 2021;384(6):533–40. https://doi.org/10.1056/NEJMoa2034545.

[19] Hall VJ, Foulkes S, Charlett A, Atti A, Monk EJM, Simmons R, et al. SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN). Lancet 2021;397(10283):1459–69. https://doi.org/10.1016/S0140-6736(21)00675-9.

[20] CDC. About COVID-19 Vaccine: requently Asked Questions about COVID-19 Vaccination. (Updated May 12, 2021) https://www.cdc.gov/coronavirus/2019-ncov/vaccines/faq.html [Accessed 2 June 2021]

[21] Zhang H, Hu Y, Jiang Z, Shi N, Lin H, Liu Y et al. Single-Cell Sequencing and Immune Function Assays of Peripheral Blood Samples Demonstrate Positive Responses of an Inactivated SARS-CoV-2 Vaccine. Available at SSRN: https://ssrn.com/abstract=3774153 or http://dx.doi.org/10.2139/ssrn.3774153.

[22] Kristiansen PA, Page M, Bernasconi V, Mattiuzzo G, Dull P, Makar K, et al. WHO International Standard for anti-SARS-CoV-2 immunoglobulin. Lancet 2021;397 (10282):1347–8. https://doi.org/10.1016/S0140-6736(21)00527-4.

[23] FDA. Convalescent Plasma. https://www.fda.gov/media/141477/download [Accessed 2 June 2021]

Taylor & Francis

Human Vaccines & Immunotherapeutics

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/khvi20

Comparison of immunogenicity and reactogenicity of inactivated SARS-CoV-2 vaccine (CoronaVac) in previously SARS-CoV-2 infected and uninfected health care workers

Ahmet Soysal, Erdem Gönüllü, Nalan Karabayır, Servet Alan, Serkan Atıcı, İsmail Yıldız, Havva Engin, Mahmut Çivilibal & Metin Karaböcüoğlu

To cite this article: Ahmet Soysal, Erdem Gönüllü, Nalan Karabayır, Servet Alan, Serkan Atıcı, İsmail Yıldız, Havva Engin, Mahmut Çivilibal & Metin Karaböcüoğlu (2021): Comparison of immunogenicity and reactogenicity of inactivated SARS-CoV-2 vaccine (CoronaVac) in previously SARS-CoV-2 infected and uninfected health care workers, Human Vaccines & Immunotherapeutics, DOI: 10.1080/21645515.2021.1953344

To link to this article: https://doi.org/10.1080/21645515.2021.1953344

Published online: 29 Jul 2021.

HUMAN VACCINES & IMMUNOTHERAPEUTICS
https://doi.org/10.1080/21645515.2021.1953344

Taylor & Francis
Taylor & Francis Group

RESEARCH PAPER

Comparison of immunogenicity and reactogenicity of inactivated SARS-CoV-2 vaccine (CoronaVac) in previously SARS-CoV-2 infected and uninfected health care workers

Ahmet Soysal[a], Erdem Gönüllü[b], Nalan Karabayır[c], Servet Alan[d], Serkan Atıcı[e], İsmail Yıldız[f], Havva Engin[g], Mahmut Çivilibal[h], and Metin Karaböcüoğlu[a]

[a]Memorial Ataşehir Hospital, Clinic of Pediatrics, İstanbul, Turkey; [b]Istanbul Health and Technology University, Department of Pediatrics, İstanbul, Turkey; [c]Medipol University Hospital, Department of Pediatrics, İstanbul, Turkey; [d]Memorial Şişli Hospital, Clinic of Infectious Diseases, İstanbul, Turkey; [e]Okan University Hospital, Department of Pediatric Infectious Diseases, İstanbul, Turkey; [f]Department of Pediatrics, Namık Kemal University School of Medicine, Tekirdağ, Turkey; [g]Memorial Ataşehir Hospital, Hospital Infection Control Unit, İstanbul, Turkey; [h]Department of Pediatrics, Memorial Bahçelievler Hospital, İstanbul, Turkey

ABSTRACT

The effects of inactivated SARS-CoV-2 vaccine (CoronaVac) on previously naturally infected individuals are unknown. This study compared immunogenicity and reactogenicity of CoronaVac in once naturally infected health-care workers (HCWs) and uninfected HCWs. All HCWs were immunized with two doses of CoronaVac (600 U/0.5 ml) intramuscularly at a 28-day interval. Adverse reactions were obtained by web-based questionnaires or telephone calls seven days after each vaccine dose. Detection of antibody levels against the receptor-binding domain (RBD) of SARS-CoV-2 spike protein was done four weeks after the second dose of the vaccine. We enrolled 103 previously naturally infected and 627 uninfected HCWs. The mean time for vaccination after the first nasopharyngeal SARS-CoV-2 positivity was 64 days (range: 15–136 days) in previously naturally infected HCWs. Among the previously naturally infected HCWs, 41 (40%) were asymptomatic, 52 (50%) had mild upper respiratory tract infections, 10 (105) had pneumonia, and only 6 (5%) were hospitalized. Any reported adverse reactions, either from the first dose or the second dose of vaccine administration, did not differ between previously infected and uninfected HCWs. Anti-RBD antibody titers were obtained in 50 (51%) of 103 previously infected HCWs and 142 (23%) of 627 uninfected HCWs. Anti-RBD antibody titers were significantly higher in HCWs with a previous natural infection (median 1220 AU/ml, range: 202–10328 AU/mL) than in uninfected HCWs (median: 913 AU/ml, range: 2.8–15547 AU/mL, p = .032). CoronaVac administration was safe and may elicit higher antibody responses in previously naturally infected individuals.

ARTICLE HISTORY
Received 20 April 2021
Revised 15 June 2021
Accepted 3 July 2021

KEYWORDS
SARS-coV-2 inactivated virus vaccine; CoronaVac; health care workers; vaccine antibody response; vaccine adverse effects; Turkey

Introduction

COVID-19 began in late 2019 and has spread worldwide and caused social and economic destruction in many countries. Health workers are among the most affected groups. Some studies reported that health-care workers who have intense and close contact with infected individuals can suffer from COVID-19 disease more than once.[1] Safe and effective COVID-19 treatments have yet to be developed, but vaccination is an effective strategy in stopping the spread of SARS-CoV-2. Several vaccines have become available for use in different parts of the world: Over 40 candidate vaccines are in human trials, and over 150 are in preclinical studies.[2]

In Turkey, the SARS-CoV-2 vaccination program started on January 11, 2021, with priority given to HCWs and then to high-risk groups. This strategy uses two doses of CoronaVac 600 U/0.5 mL (Sinovac Life Science Co, Ltd, Beijing, China) given 28 days apart intramuscularly.[3] The BNT162b2 vaccine (Pfizer-BioNTech) was later introduced to the immunization program with two doses given at four-week intervals.[3] The total number of vaccines given in Turkey is 18,724,856; 7,619,467 have received the second dose.[3] Previously, SARS-CoV-2 infected people are thought to have protective immunity and memory responses for at least six months.[4] However, the ideal vaccination time and regimens have not yet been clarified in previously infected individuals. It is also reasonable for such individuals to delay any vaccine receipt for a few months after infection to allow others to get vaccinated sooner as the risk of reinfection appears extremely low in this period. The USA Centers for Disease Control and Prevention (CDC) also suggest that individuals who received monoclonal antibodies or convalescent plasma for COVID-19 should delay vaccination for at least 90 days from the time of treatment.[5] The Turkish Ministry of Health recommended SARS-CoV-2 vaccination at least one month after COVID-19 infection in HCWs and six months later in high-risk group individuals. Individuals with a history of SARS-CoV-2 may also be more likely to experience local and systemic adverse reactions.[5,6] However, the responses to SARS-CoV-2 inactivated virus vaccine (CoronaVac, Sinovac Life Science Co., Ltd, Beijing, China) in previously naturally infected individuals have not yet been assessed in clinical trials. Therefore, this study compared antibody response and adverse reactions between previously

CONTACT Ahmet Soysal drahmetsoysal20@gmail.com Memorial Ataşehir Hospital, Clinic of Pediatrics, Vedat Gunyol Street 28, İstanbul 34758, Turkey

Table 1. Demographic and clinical features of study population.

	Previously SARS-CoV-2 infected n = 103	SARS-CoV-2 uninfected n = 627	P value
Age, median (range), years	36 (22–68)	41 (22–72)	<.001
Sex			
Male	40 (37%)	247 (39%)	.9
Female	63 (63%)	380 (61%)	
Clinic severity			
Asymptomatic	41 (40%)	-	
URTI	52 (50%)	-	
Pneumonia	10 (10%)	-	
Hospitalization	6 (5%)	-	
Days from NP SARS-CoV-2 PCR + to vaccination mean (range)	64 (15–136)	-	
Days from 2nd dose vaccination to collecting blood for antibody mean; (range)	28 days (13–34)	28 days (15–36)	.8
Any adverse Reactions after 1st dose of vaccine	44 (42%)	309 (43%)	.15
Any adverse Reactions after 2nd dose of vaccine	34 (35%)	214 (34%)	.25
Number of vaccinated individuals with available antibody result	50 (51%)	142 (23%)	-
Number of vaccinated subjects with undetectable antibody titers	0 (0%)	2 (%1)	-

SARS-CoV-2 naturally infected and uninfected health-care workers (HCWs) after two doses of SARS-CoV-2 vaccine (CoronaVac) administration.

Materials and methods

This study was a nested case–control analysis of 103 HCWs with previous natural SARS-CoV-2 infection during the last four months before administering the first dose of SARS-CoV -2 inactivated virus vaccine (CoronaVac, Sinovac Life Science Co, Ltd, Beijing, China); there were also 627 infection-naive HCWs. All work was done between January 11 and February 25, 2021. This study was done at Memorial Istanbul Ataşehir Hospital and Memorial Istanbul Şişli Hospital. To investigate vaccine-related adverse reactions, we made an online web-based questionnaire using The Turkish Pediatric Workshop telegram group.[7] Clinical features and antibody titers results were obtained from participating hospitals' infection control unit records. Vaccine-related adverse reactions were collected seven days after each vaccine-dose administration via web-based questionnaires. Antibody titers were measured four weeks after the second dose of the vaccine. Antibodies against the receptor-binding domain (RBD) of SARS-CoV-2 spike protein were measured with a SARS-CoV -2 IgG II Quant Reagent Kit (Abbott Ireland Diagnostics Division, Finisklin Business Park, Sligo, Ireland).

CoronaVac is an inactivated virus vaccine with an alum adjuvant. The SARS-CoV-2 strain CN2 was extracted from bronchoalveolar lavage (BAL) of a hospitalized patient in Wuhan, cultured in Vero cells, harvested, inactivated using β-propiolactone, and purified before being absorbed into aluminum hydroxide.[8] Each 0.5-mL vaccine vial contains 600 SU SARS-CoV-2 antigens, sodium chloride (9 mg/ml), disodium hydrogen phosphate (1.16 mg/ml), monosodium hydrogen phosphate, sodium hydroxide, and sterile water. All HCWs received two doses of CoronaVac at least 28 days apart, and blood was drawn for detection of anti-RBD antibody four weeks after the second dose of the vaccine. All HCWs provided informed consent. This study was approved by the COVID-19 scientific research commission of the Turkish Ministry of Health and ethically approved by the Istanbul Memorial Şişli Hospital ethics committee. Statistical analysis was performed with jamovi (version 1.6, computer software retrieved from https://jamovi.org.) Antibody titers between groups were tested using the two-tailed Mann-Whitney U-test, Student's t-test, and Pearson $\chi 2$ test for categorical and continuous variables. A *P*-value <0.05 was considered significant.

Results

Of the 730 HCWs enrolled in the survey, 103 (14%) HCWs had a previous laboratory-confirmed mild or asymptomatic SARS-CoV-2 infection as diagnosed with positive nasopharyngeal aspiration (NP) swab PCR (only one HCW had a negative PCR result but positive anti-SARS-CoV2 IgM antibody); 627 (86%) HCWs were previously uninfected as shown by PCR. Demographic and clinical features of the study population are shown in Table 1. Among the previously naturally SARS-CoV -2 infected HCWs, 41 (40%) of them were asymptomatic, 52 (50%) had mild upper respiratory tract infection, 10 (10%) of them had pneumonia, and only 6 (5%) were hospitalized. None of the previously naturally SARS-CoV-2 infected HCWs died. The mean time for vaccination from the first nasopharyngeal SARS-CoV-2 positivity was 64 days (range: 15–136 days) in previously naturally SARS-CoV-2 infected HCWs. None of the HCWs received steroids or other immune-suppressive drugs for the treatment of SARS-CoV-2 infection.

Any reported adverse reactions – whether from the first or second dose of vaccine administration – did not differ between previously infected and uninfected HCWs (Table 1). The most common self-reported vaccine-related adverse effects after the first dose of the vaccine were local injection site pain (41%), myalgia (19%), and headache (13%) in previously uninfected HCWs; injection site pain (44%) and myalgia (13%) were seen in once-infected HCWs. The most common self-reported vaccine-related adverse effects after the second dose of the vaccine were local injection site pain (26%), headache (12%), and myalgia (3%) in previously uninfected HCWs, and injection site pain (30%), and myalgia (3%) in previously infected HCWs. Self-reported adverse reactions for the second dose were lower in both groups than the first dose (Table 1). Interestingly, sleepiness was reported after the first dose of vaccine in 14% of previously infected HCWs and 16% of previously uninfected HCWs; the rate of sleepiness

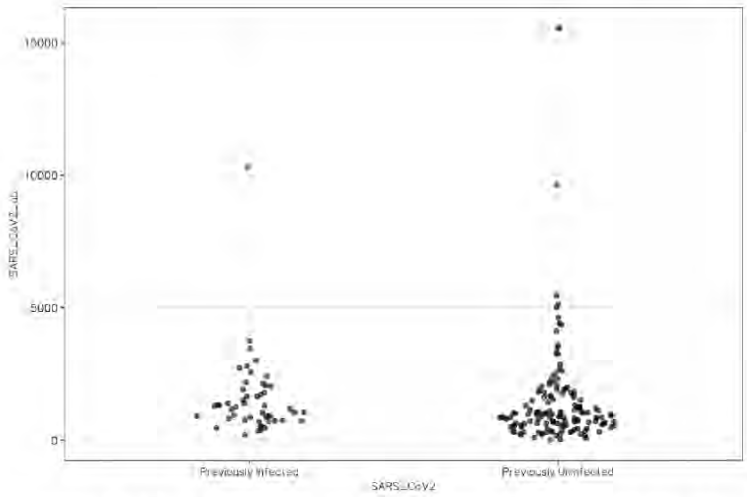


Figure 1. Anti-SARS-CoV-2 antibody responses after 2 doses of vaccine in health care workers concerning previous infection status. Anti-RBD antibody (Arbitrary unit per ml)

decreased to 7% in previously infected HCWs and decreased to 10% in uninfected HCWs after the second dose. The reported sleepiness rate, whether after the first dose or second dose of the vaccine administration, did not differ between previously infected and uninfected HCWs ($p > .05$, respectively).

The study included 103 previously infected HCWs and 627 uninfected HCWs. Anti-RBD-antibody (SARS-CoV-2 IgG) titers were obtained in 50 (51%) of 103 previously infected HCWs and 142 (23%) of 627 uninfected individuals; 190 (98%) of seroprevalent patients reached an assay detectable response (SARS-CoV-2 IgG index value ≥50 AU/mL). Only two (2%) HCWs who were 53 and 52 years of age with no previous-SARS-CoV-2 infection had an undetectable antibody level despite vaccination. Anti-RBD antibody titers were significantly higher in HCWs with previous natural infection (median 1220 AU/ml, range: 202–10328 AU/mL) than in uninfected HCWs (median: 913 AU/ml, range: 2.8–15547 AU/mL, $p = .032$) (Figure 1).

Discussion

To the best of our knowledge, this is the first study to investigate reactogenicity and immunogenicity of inactivated SARS-CoV-2 vaccine (CoronaVac) in previously naturally infected individuals. Studies with inactivated SARS-CoV-2 vaccine (CoronaVac, Sinovac Life Science Co., Ltd., Beijing, China) have shown that most adverse reactions were mild. The most common symptom was injection-site pain, which agrees with previous studies. Previously, phase 1–2 clinical trials of CoronaVac among healthy adults aged 18–59 years showed that the vaccine was well tolerated, and seroconversion rates were 97–100% 28 days after the second dose of vaccine depending on the amount of antigen.[8]

Our study is in parallel with phase 1 and 2 studies of inactivated SARS-CoV-2 vaccine (CoronaVac, Sinovac Life Science Co., Ltd., Beijing, China); 98% of vaccinated HCWs had a detectable antibody response. This study's main finding is that HCWs with previous SARS-CoV-2 infection had a higher antibody titer response to two doses of inactivated SARS-CoV-2 vaccine (CoronaVac, Sinovac Life Science Co, Ltd, Beijing, China) than those who were not previously infected. The median anti-RBD antibody titers were significantly higher in HCWs with previous natural infection (median 1220 AU/ml, range: 202–10328 AU/mL) than in uninfected HCWs (median: 913 AU/ml, range: 2.8–15547 AU/mL, $p = .032$).

To the best of our knowledge, there is no reported research either investigating the safety or immunogenicity of inactivated SARS-CoV-2 vaccine (CoronaVac, Sinovac Life Science Co., Ltd., Beijing, China) in previously naturally infected individuals. As a result, we cannot compare our findings to the literature. We examined studies done with other SARS-CoV-2 vaccines: Higher antibody titers after a single dose of mRNA vaccines were seen in previously naturally infected HCWs in many studies.[6,9–14]

Prendecki et al. reported that anti-S titers were significantly higher in HCWs with previous natural infection than in infection-naive HCWs after a single-dose of BNT 161b2 mRNA vaccine (Pfizer-BioNTech, Mainz, Germany) (median 16353 AU per mL [IQR 4741–28 581] *vs.* 615 · 1 AU/mL (286 · 4–1491)) [10]. Manisty et al. also compared a single dose of BNT162b2 mRNA COVID-19 vaccine (Pfizer-BioNTech, Mainz, Germany) responses in HCWs.[10] They reported that among previously uninfected, seronegative individuals, anti-S titers after one vaccine dose were comparable to peak anti-S titers in individuals with a previous natural infection who had not yet been vaccinated. Among those with previous SARS-CoV-2 infection, vaccination increased anti-S titers more than 140-fold from peak pre-vaccine levels. This increase appears to be at least one order of magnitude greater than values reported after a conventional prime-boost vaccine strategy in previously uninfected individuals.[10]

Saadat et al. also investigated antibody responses after single-dose mRNA vaccines (either the Pfizer-BioNTech or Moderna vaccine) in 17 antibody-negative subjects, 16 asymptomatic SARS-CoV-2-infected subjects, and 26 symptomatic

SARS-CoV-2-infected HCWs. HCWs with previous COVID-19 infection had higher antibody titer responses to a single dose of mRNA vaccines than those who were not previously infected based on laboratory-confirmed serology testing.

Antibody titers started peaking at seven days and achieved higher titers and neutralization rates in 14 days than antibody-negative volunteers.[11] Bradley et al. determined antibody levels at baseline and three weeks after the first dose of the BNT162b2 SARS-CoV-2 mRNA vaccine (Pfizer-BioNTech, Mainz, Germany) in 36 HCWs who received laboratory confirmation of SARS-CoV-2 infection 30 to 60 days before they received the vaccine as well as 152 HCWs without a history of SARS-CoV-2 infection.[12] They showed that three weeks after a single vaccination, HCWs with recent SARS-CoV-2 infection or seropositive status had higher antibody levels to SARS-CoV-2 antigens and higher levels of antibodies with neutralizing characteristics than those without a history of infection.[12]

Krammer et al. investigated antibody responses after mRNA vaccines (BNT162b2 [Pfizer] and mRNA-1273 [Moderna]) in 67 SARS-CoV-2 seronegative individuals and 43 seropositive individuals.[6] They reported that the antibody titers of vaccines with preexisting SARS-CoV-2 antibody were 10 to 45 times as high as those vaccinated without preexisting antibodies at the same time points after the first vaccine dose. Seropositive patients also exceeded the median antibody titers measured in participants without preexisting antibodies after the second vaccine dose by more than a factor of 6.[6] In addition, Ebinger et al. compared antibody responses to BNT162b2 (Pfizer–BioNTech) mRNA vaccine in individuals with previous SARS-CoV-2 infection (n = 35) versus infection-naive (n = 228) individuals.[13] They reported that individuals previously infected with SARS-CoV-2 developed vaccine-induced antibody responses after a single dose of the BNT162b2 (Pfizer–BioNTech) mRNA vaccine that was similar to the antibody responses seen after a two-dose vaccination course administered to infection-naive individuals.[13]

In contrast, Tauzin et al. investigated humoral and T cell immune responses in cohorts of SARS-CoV-2 naive (n = 16) and naturally infected individuals (n = 16) prior and three weeks after the BNT162b2 (Pfizer–BioNTech) mRNA vaccine. They found that no neutralizing activity was seen in SARS-CoV-2-naive individuals three weeks after the first dose of vaccine. They still detected strong anti-RBD and spike antibodies with F_c-mediated effector functions and cellular responses dominated by the $CD4^+$ T cell component. Moreover, after a single dose of the vaccine, a significant increase in preexisting humoral immunity, neutralization, and all T-cell responses were observed in SARS-CoV-2 naturally infected individuals.[14]

Covaxin was developed by the Indian pharmaceutical company Bharat Biotech in collaboration with the Indian Council of Medical Research (a government-funded biomedical research institute), and its subsidiary the National Institute of Virology; 800 participants have been enrolled in ongoing phase III trials since November 25, 2020. Bharat Biotech released interim efficacy data on March 3, 2021, which showed a clinical efficacy of 81%.[15]

This study shows that any adverse reactions after inactivated SARS-CoV-2 vaccine (CoronaVac, Sinovac Life Science Co, Ltd, Beijing, China) administration did not differ between previously infected and uninfected individuals. Healthy adults aged 18–89 years easily tolerated the vaccine in phase 1–2 clinical trials of inactivated SARS-CoV-2 vaccine (CoronaVac, Sinovac Life Science Co., Ltd., Beijing, China). In the phase 1 trial, 38% of subjects in the high-dose vaccine group reported adverse reactions. The most common symptom was injection site pain and the most adverse reactions were mild (grade 1) similar to our observations. The literature shows that previously infected individuals experienced significant post-vaccine symptoms more frequently than infection-naïve individuals after the first dose of BNT162b2 (Pfizer–BioNTech) mRNA vaccine. This difference was not observed after the second dose; naive individuals reported higher reactogenicity than previously infected individuals.[13] Krammer et al. reported higher frequencies of any adverse reactions and systemic side effects after mRNA vaccines (BNT162b2 [Pfizer] and mRNA-1273 [Moderna]) in vaccine recipients with preexisting immunity.[6] Prendecki et al., Manisty et al., Saadat et al., and Bradly et al. did not mention adverse vaccine reactions in their reports.[9–12]

Our study's limitations are a small sample size, lack of pre-vaccination antibody titers of participants, lack of investigation of cellular immune responses, demonstration of vaccine efficacy, and potential enrollment bias. Because of ongoing worldwide vaccine shortages, this study's results might lead to suggestions on a single-dose vaccination strategy for those with previous SARS-CoV-2 infection but this needs further study.

In conclusion, we showed that the CoronaVac vaccine elicits antibody responses in both SARS-CoV-2-uninfected and previously naturally infected individuals; the median antibody responses were higher in previously infected individuals. Furthermore, there was no difference in vaccine-related adverse reactions between previously infected and uninfected individuals either in the first or second dose. However, further study is needed to clarify if a single-dose of CoronaVac is sufficient for previously infected individuals.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

ORCID

Erdem Gönüllü http://orcid.org/0000-0002-6833-5646
Nalan Karabayır http://orcid.org/0000-0002-8003-1952
Servet Alan http://orcid.org/0000-0002-8228-6350
İsmail Yıldız http://orcid.org/0000-0002-4990-0216
Metin Karaböcüoğlu http://orcid.org/0000-0003-2854-0562

References

1. Atici S, ÖF E, Yildiz MS, Şikgenç MM, Güzel E, Soysal A. Symptomatic recurrence of SARS-CoV-2 infection in healthcare workers recovered from COVID-19. J Infect Dev Ctries. 2021 Jan 31;15(1):69–72. doi:10.3855/jidc.14305.
2. World Health Organization. Draft landscape of COVID-19 candidate vaccines. [accessed 2020 Oct 20]. https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines .

3. The Turkish Ministry of Health Covid19 information page. [accessed 13 Apr 2021]. https://covid19asi.saglik.gov.tr/?_Dil=2 .
4. Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, Grifoni A, Ramirez SI, Haupt S, Frazier A, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science. 2021 Feb 5;371(6529):eabf4063. doi:10.1126/science.abf4063.
5. Interim clinical considerations for use of mRNA COVID-19 vaccines currently authorized in the United States. [accessed 2021 Mar 3]. https://www.cdc.gov/vaccines/covid-19/info-by-product/clinical-considerations.html .
6. Krammer F, Srivastava K, Alshammary H, Amoako AA, Awawda MH, Beach KF, Bermúdez-González MC, Bielak DA, Carreño JM, Chernet RL, et al. Antibody Responses in Seropositive Persons after a Single Dose of SARS-CoV-2 mRNA Vaccine. N Engl J Med. 2021 Apr 8;384(14):1372–74. doi:10.1056/NEJMc2101667.
7. Gönüllü E, Soysal A, Can İ, Tutak E, Tunç T, Yıldız İ, Yeşilbaş O, Öner N, Anarat A, Soysal FG, et al. The use of social network in daily pediatric practice and education: Turkish pediatric atelier. Int J Pediatr. 2020 Sep 21;2020:7301309. doi:10.1155/2020/7301309.
8. Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, Han W, Chen Z, Tang R, Yin W, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021 Feb;21(2):181–92. doi:10.1016/S1473-3099(20)30843-4.
9. Prendecki M, Clarke C, Brown J, Cox A, Gleeson S, Guckian M, Randell P, Pria AD, Lightstone L, Xu XN, et al. Effect of previous SARS-CoV-2 infection on humoral and T-cell responses to single-dose BNT162b2 vaccine.
10. Manisty C, Otter AD, Treibel TA, McKnight Á, Altmann DM, Brooks T, Noursadeghi M, Boyton RJ, Semper A, Moon JC, et al. Antibody response to first BNT162b2 dose in previously SARS-CoV-2-infected individuals. Lancet. 2021 Mar 20;397(10279):1057–58. doi:10.1016/S0140-6736(21)00501-8.
11. Saadat S, Rikhtegaran Tehrani Z, Logue J, Newman M, Frieman MB, Harris AD,Sajadi MM. Binding and neutralization antibody titers after a single vaccine dose in health care workers previously infected with SARS-CoV-2. JAMA. 2021 Apr 13;325(14):1467–69. doi:10.1001/jama.2021.3341.
12. Bradley T, Grundberg E, Selvarangan R, LeMaster C, Fraley E, Banerjee D, Belden B, Louiselle D, Nolte N, Biswell R, et al. Antibody responses after a single dose of SARS-CoV-2 mRNA vaccine. N Engl J Med. 2021 May 20;384(20):1959–61. doi:10.1056/NEJMc2102051.
13. Ebinger JE, Fert-Bober J, Printsev I, Wu M, Sun N, Prostko JC, Frias EC, Stewart JL, Van Eyk JE, Braun JG, et al. Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2. Nat Med. 2021 Apr 1;27:981–84. doi:10.1038/s41591-021-01325-6.
14. Tauzin A, Nayrac M, Benlarbi M, Gong SY, Gasser R, Beaudoin-Bussières G, Brassard N, Laumaea A, Vézina D, Prévost J et al. A single BNT162b2 mRNA dose elicits antibodies with Fc-mediated effector functions and boost pre-existing humoral and T cell responses. bioRxiv [Preprint]. 2021 Mar 18:2021.03.18.435972.
15. Thiagarajan K. What do we know about India's Covaxin vaccine? BMJ 2021 Apr 20;373:n997. doi:10.1136/bmj.n997.

1.9. CoronaVac induz produção de anticorpos específicos contra as principais proteínas do vírus SARS-CoV-2

Um estudo brasileiro publicado na revista Diagnostic Microbiology and Infectious Disease identificou alta produção de anticorpos IgG específicos contra a proteína Spike (S) e contra a proteína nucleocapsídeo (N) do SARS-CoV-2 em profissionais da saúde vacinados com a CoronaVac, com e sem infecção prévia de Covid-19, e com e sem comorbidades. Essas são as proteínas mais importantes do vírus, que induzem maior resposta imune.

A análise, publicada em novembro, foi conduzida por cientistas da Universidade Federal do Paraná (UFPR) e do Centro Nacional de Pesquisa em Energia e Materiais de Campinas. Os 133 voluntários eram profissionais do Complexo Hospital de Clínicas da UFPR, de Curitiba, com idades de 25 a 59 anos, sendo nove imunossuprimidos e 124 sem comorbidades. Os indivíduos também foram divididos em outros dois grupos: que apresentaram sorologia positiva para Covid-19 antes da vacinação (16) e que nunca tiveram a doença (117).

Uma produção robusta de anticorpos IgG específicos para a proteína S, responsável pela entrada do vírus nas células humanas, foi detectada em 97% do total de participantes duas semanas após a segunda dose. Além disso, 52% dos indivíduos apresentaram anticorpos IgG contra a proteína N – isso porque a CoronaVac é uma vacina de vírus inativado capaz de promover uma resposta imune mais ampla, não restrita a uma única proteína.

Os níveis de anticorpos produzidos foram semelhantes, independente do participante já ter tido a doença ou não. Nos indivíduos imunossuprimidos, em geral, a resposta imune também foi similar ao grupo sem comorbidades.

Os pesquisadores chamam a atenção para as taxas de soroconversão observadas para a proteína N, a mais conservada e estável do vírus. "Como essa proteína apresenta um baixo nível de mutações, anticorpos específicos para essa proteína podem ser viáveis no combate às variantes, que possuem um alto número de mutações na proteína S", afirmam. No entanto, reforçam que mais estudos precisam ser feitos para entender o efeito protetor de anticorpos específicos contra outras proteínas do vírus.

Publicado em: 19/11/2021

Artigo

Diagnostic Microbiology and Infectious Disease 102 (2022) 115597

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Original Article

Dynamic of humoral response to SARS-CoV-2 anti-Nucleocapsid and Spike proteins after CoronaVac vaccination

Lucas Bochnia-Bueno[a,b], Sergio Monteiro De Almeida[a], Sonia Mara Raboni[a,b], Douglas Adamoski[c], Ludmilla Louise Moreira Amadeu[a], Suzana Carstensen[a], Meri Bordignon Nogueira[a,*]

[a] Virology Laboratory, Federal University of Paraná, Curitiba, Paraná, Brazil
[b] Post-Graduate Program in Microbiology, Parasitology and Pathology, Federal University of Paraná, Curitiba, Paraná, Brazil
[c] Brazilian Biosciences National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, Sao Paulo, Brazil

ARTICLE INFO

Article history:
Received 6 July 2021
Revised in revised form 12 November 2021
Accepted 14 November 2021
Available online 19 November 2021

Keywords:
Vaccine
Immunization
Public health
Immunoglobulin G
CoronaVac
Pandemic

ABSTRACT

This study aimed to calculate the seroconversion rate and IgG antibody dynamic range of the CoronaVac vaccine in healthcare workers (HCWs) after immunization. Serum samples from 133 HCWs from Southern Brazil were collected 1 day before (Day 0) and +10, +20, +40, + 60, +110 days after administering the vaccine's first dose. Immunoglobulin G (IgG) was quantified using immunoassays for anti-N-protein (nucleocapsid) antibodies (Abbott, Sligo, Ireland) and for anti-S1 (spike) protein antibodies (Euroimmun, Lübeck, Germany). Seroconversion by day 40 occurred in 129 (97%) HCWs for the S1 protein, and in 69 (51.87%) HCWs for the N protein. An absence of IgG antibodies (by both methodologies), occurred in 2 (1.5%) HCWs undergoing semi-annual rituximab administration, and also in another 2 (1.5%) HCWs with no apparent reason. This study showed that CoronaVac has a high seroconversion rate when evaluated in an HCW population.

1. Introduction

By July 5, 2021, approximately 1 year after the beginning of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, confirmed cases of infection worldwide numbered 183,560,151 people, including 3,978,581 deaths (World Health Organization (WHO) 2021). After the description of this new human coronavirus in December 2019, there was a global effort by researchers, public and private companies in the search for an effective vaccine to control this pandemic (Angeli et al., 2021; Golob et al., 2021; Kumar et al., 2021). These studies resulted in late 2020, with the first doses of immunization in the population, and there are currently 2,988,941,529 doses of the vaccine administered until July 5, 2021 (WHO, 2021).

Many SARS-CoV-2 proteins can induce an immune response, amongst them: M (membrane), E (envelope), N (nucleocapsid), and S (spike) (Zeng et al., 2020). However, the S and N proteins are the most responsive to infection, which induces high titers of anti-SARS-CoV-2 IgM and IgG antibodies. S protein has been more studied for vaccines because it participates in the virus entry mechanism through the connection of the S1 region receptor-binding domain (S1-RBD) in virus particles with the angiotensin-converting enzyme 2 (ACE 2) in the host cell (Barchuk et al., 2021; Saelens and Schepens, 2021). Then, the antibodies binding in this region can cause viral neutralization. Both S and N proteins have also been used for diagnosis, S protein is more specific despite being a more variable portion. In contrast, N protein is a more preserved region, including high homology with N protein SARS-CoV (>90%), but both may have false-positive results (Jiang et al., 2020). To evaluate the neutralization antibody activity, the gold-standard assay is the plaque reduction neutralization test (PRNT) that involves the measurement of the ability of patient sera to prevent infection (Murray et al., 2021). However, since this assay is time-consuming and requires higher levels of biological safety, multiple groups proposed anti-RBD ELISA assays as a reliable tool to predict neutralization (Murray et al., 2021; Padoan et al., 2021; Papenburg et al., 2021).

Worldwide efforts resulted in several vaccines against SARS-CoV-2 with distinct antigen platforms systems (nonreplicating viral vector, protein subunit, inactivated virus, and mRNA), with the main antigenic focus on S protein (Golob et al., 2021; Kumar et al., 2021).

The vaccination in Brazil started with CoronaVac (Sinovac Life Sciences, Beijing, China) in January 2021, and until June 2021, 2 other vaccines come into use in the country. However, CoronaVac (Sinovac Life Sciences, Beijing, China) remains the most administered in Brazilian territory (Brasil, Ministério da Saúde 2021), using the inactivated

* Corresponding author. Tel.: +55-41-3360-7974; fax: +55-41-3360-1811
E-mail address: meribordignon.nogueira@gmail.com (M.B. Nogueira).

https://doi.org/10.1016/j.diagmicrobio.2021.115597
0732-8893/

virus as a component of the vaccine (Golob et al., 2021; Kumar et al., 2021). In phase I/II studies, this vaccine was safe, tolerable, presented high immunogenicity, and had uncommon adverse reactions. A similar response was observed for both tested concentrations (3 μg and 6 μg), and 97% of seroconversion occurred in the participants with 18 to 59 ages (Padoan et al., 2021). In phase III trials, carried out with health care workers, this vaccine presented 50.7%, 83.7%, and 100% efficacy against symptomatic disease, cases requiring assistance, and severe cases, respectively (Zhang et al., 2021a, 2021b). Phase III also tested some serum samples against the B.1.1.28, gamma (P.1), and zeta (P.2) variants, showing great antibody response (Palacios et al., 2021).

As the vaccine has been administered to people with different ethnicities, comorbidities, and ages, the results of pre-approval clinical trials for its use may not perfectly reflect the response to the vaccine. Thus, vaccine response analyses, either by seroconversion or by neutralizing antibody titration, are essential to assess the possible impacts of this immunization on the population and must be monitored so that the humoral response time can be defined. In this context, this study aimed to identify the seroconversion rate and antibody dynamic range after vaccination with SARS-CoV-2 (CoronaVac) in healthcare workers (HCWs) 40 days after its application.

2. Methods

2.1. Participants

In total, 170 participants were recruited at the Complexo Hospital de Clínicas, UFPR, Clinical Laboratory, Curitiba, Brazil, during the vaccination of HCWs in this city. The Institutional Ethical Committee approved the study (CAAE: 31687620.2.0000.0096), and all participants signed their consent.

The inclusion criteria were as follows: answering the questionnaire, being vaccinated with 2 doses of CoronaVac, and providing serum samples. Fourteen participants were excluded because they did not complete the questionnaire. In addition, 7 participants took another vaccine, 1 participant did not have the second dose, and 15 participants did not provide a sample on days 0 (previous vaccination) or +40 (post-vaccination) (Fig. 1).

Serum samples of 133 healthcare workers included in this study were collected on days 0 (previous first dose application), +10, +20, +40, +60, and +110 after the first dose. On day 0 and +40, 133 serum samples were analyzed, and on day +10, +20, +60 and +110, 123, 119, 114 and 132 serum samples were analyzed, respectively. All samples were stored at −20 °C until analysis.

The participants were divided into 2 groups based on day 0 serology according to anti-spike-1 (anti-S1) immunoglobulin G (IgG) (Dutta et al., 2020, Fergie and Srivastava, 2021, Zeng et al., 2020): *reactive* (n = 16) and *nonreactive* (n = 117). The participants were also sorted according to the presence of comorbidities into 2 divisions: *immunosuppressed* (n = 9) or *not* (n = 124) (Fig. 1; Table 1). The immunosuppressed group consisted in participants who presented comorbidity associated with compromised humoral or cellular immune response or those who used immunosuppressive drugs, such as HIV infection, use of chemotherapy or steroids (prednisone at a dose of 20 mg/day or equivalent).

2.2. Seroconversion evaluation

Semi-quantitative assays were performed to detect anti-SARS-CoV-2 IgG. For all serum samples, assays used the Chemiluminescent Microparticle Immunoassay (CMIA) Architect-I System for anti-nucleocapsid protein (anti-N) IgG (Abbott, Sligo, Ireland). Additionally, for serum samples from days 0, +40 and +110, assays used the Enzyme-Linked Immunosorbent Assay (ELISA) for IgG anti-S1 spike-protein receptor-binding domain (RBD) (Euroimmun, Lübeck, Germany).

Samples were tested in duplicate, following the manufacturer's instructions. Results with a variation coefficient greater than 15.0% were repeated.

2.3. Statistical analysis

According to the distribution of seroconversion at day +40, the category variables were evaluated using Pearson's chi-squared test with Yates' continuity correction. The age variable was evaluated using the Wilcoxon signed rank sum test with continuity correction. Samples paired over time were evaluated using the Friedman ANOVA test (as implemented in the rstatix package), followed by the Wilcoxon signed rank test as a post hoc pairwise comparison. For samples without multiple observations over time, the Wilcoxon signed rank test was used. All statistical analyses were performed using R (R Core Team). *P* values less than 0.05 were considered significant.

3. Results

3.1. Seroconversion to S1 protein

Robust production of anti-S1-protein IgG was observed by day +40 in 129 (97%) HCW participants by the index test result. Although the reactive (Fig. 2D) and nonreactive (Fig. 2B) groups had different average index values for S1-protein IgG on day 0 ($P < 0.0001$), on day +40, the average index between the groups was not significantly different ($P = 0.3704$).

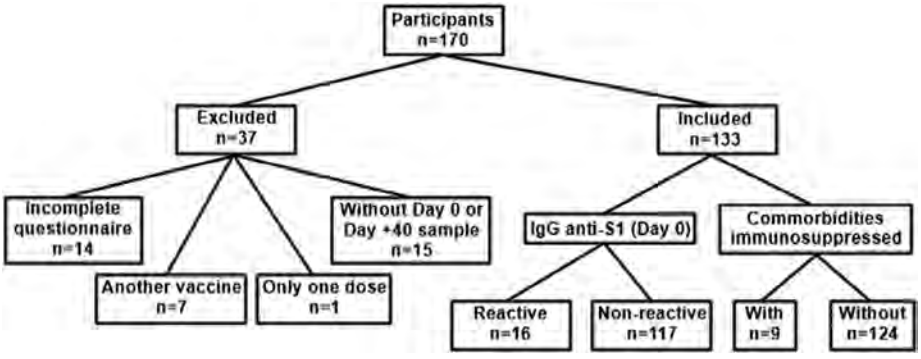


Fig. 1. Participants included and excluded in the study and division of groups for analysis. Comorbidities (immunosuppressive) included: Immunosuppressive drugs use, Crohn's disease, bariatric surgery, HIV and Diabetes.

Table 1
Demographics characteristics of participants included in the study for each respective group.[a]

	IgG Anti-S1 (Day 0)			Comorbidities immunosuppressive[b]		
	Reactive n (%)	Nonreactive n (%)	*P* value	With n (%)	Without n (%)	*P* value
Total	16	117		9	124	
Female	13 (81.25)	93 (79.49)	1.0000	6 (66.67)	100 (80.64)	0.5636
Median Age (IQR)	44 (25.25–52.75)	49 (39.50–53.50)	0.2225	51 (45.50–54.50)	48 (38.25–53.75)	0.2297

[a] Information on the handling of special cases: 2 immunosuppressed (Rituximab 1400 mg/semiannually), 1 myasthenia gravis (Pyridostigmine 120 mg/day), 1 Crohn's disease ostomized 22 years ago (Azathioprine 100 mg/day), 2 participants with bariatric surgery (11 and 12 years), and 1 HIV+ (Tenofovir 300 mg, Lamivudine 300 mg + Dolutegravir 50 mg/day; $CD4^+$ 541/μL).

[b] Comorbidities (immunosuppressive) included: Immunosuppressive drugs use, Crohn's disease, bariatric surgery, HIV and Diabetes. The patient with Myasthenia gravis is not included here because the treatment used was not immunosuppressive.

3.2. Seroconversion to N protein

Distributing the data in the division of groups is possible to observe no significant production of the anti-N-protein IgG in nonreactive group participants 10 days after the first vaccine dose ($P = 0.5027$; Fig. 2A), and although there was a statistical difference in the sample on day +20 ($P < 0.0001$), there was no apparent seroconversion at that time. By contrast, there was a marked increase in N-protein IgG levels in 69 (51.87%) participants on day +40 (Fig. 2A). A significant difference was also observed in the average index for this antibody between the reactive (Fig. 2C) and nonreactive groups (Fig. 2A): day 0 ($P < 0.0001$) and day +40 ($P = 0.0657$).

3.3. Combined response

In the nonreactive group, better-developed antibody responses were observed for N and S1 proteins ($P < 0.0001$; Fig. 2A, B), while in the reactive group, the antibody response showed a significant

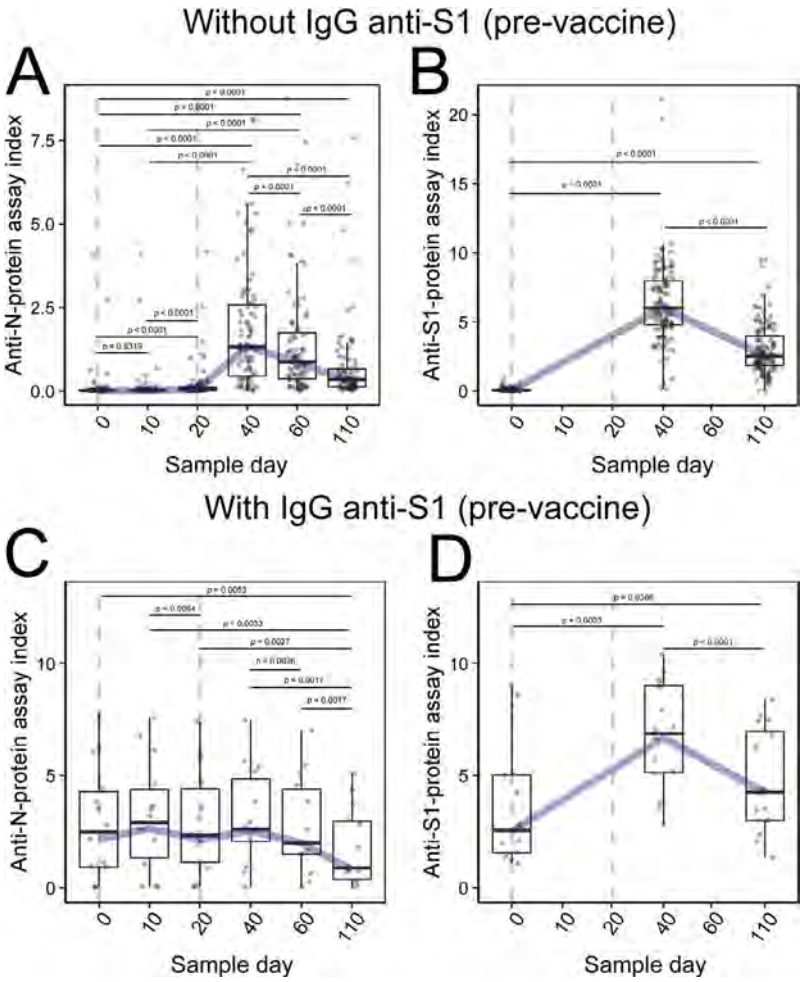


Fig. 2. Antibody rates in the S1-protein IgG seroconverted/not seroconverted groups at day 0. Boxplot graph presents median (line dividing the box), interquartile range (box), maximum value (line above the box), and minimum value (line below the box). The line connecting the boxes represents the trend of the data. The dotted line represents the days of the vaccine application (2 doses). (A) N-protein IgG evaluation in S1-antibody nonreactive participants at day 0. (B) S1-protein IgG evaluation in S1-protein IgG nonreactive participants at day 0. (C) N-protein IgG evaluation in S1-protein IgG reactive participants at day 0. (D) IgG anti-S1 protein evaluation in anti-S1 protein IgG reactive participants at day 0.

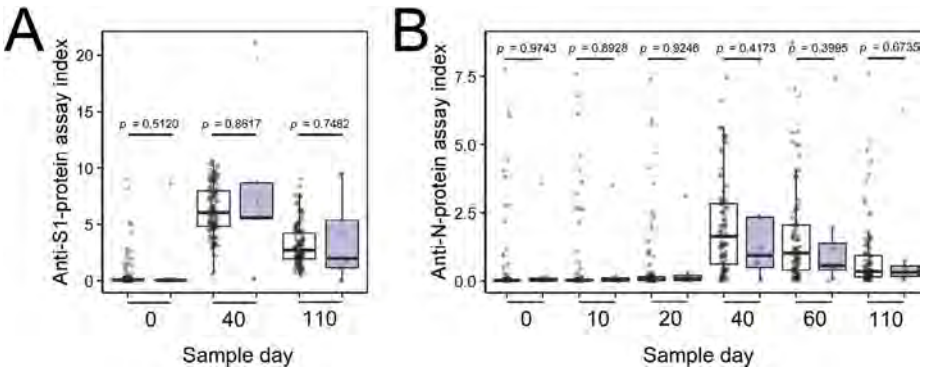


Fig. 3. Antibody rates for participants with and without immunosuppression. White boxes indicate nonimmunosuppressed participants. Gray boxes indicate immunosuppressed participants. (A) S1-protein IgG evaluation. (B) N-protein IgG evaluation.

difference ($P < 0.0001$) only for antibodies against S1 protein (Fig. 2D), increasing the level of circulating humoral response. No significant changes were observed in IgG anti-N protein analysis for the reactive group at days +10, +20, and +40 ($P = 0.2231$). The antibody index for IgG anti-N and anti-S1 presented at day +40 approximated mean of 2.0 and 6.0, respectively.

Comorbidities were reported by some HCWs, including Crohn's disease, prior bariatric surgery, HIV+, or diabetes. In general, the participants with comorbidities responded to the vaccine similarly to participants without any comorbidities (Fig. 3). However, 2 cases in the immunosuppressed group did not undergo seroconversion. Furthermore, 2 other HCWs (not in the immunosuppressed group) did not seroconvert by day +40; both had no apparent cause. These 4 HCWs without seroconversion were re-evaluated at +60 and +110 days. One participant presented seroconversion of the S1 protein in a sample of +60 days (Fig. 4).

In the anti-S1 reactive group on day 0, 6 (37.50%) participants did not have a previous SARS-CoV-2 diagnosis, possibly due to asymptomatic infection. Furthermore, in the anti-S1 nonreactive group, 7 (5.98%) participants had symptoms suggestive of SARS-CoV-2 (fever, dry cough, tiredness, loss of taste or smell, aches and pains, headache, sore throat, nasal congestion, red eyes, diarrhea, or a skin rash) (WHO, 2021), although we did not have information about nasopharyngeal RT-PCR or immunological rapid-test detection. Demographic data according to immunologic response and comorbidities, are shown in Table 1.

3.4. Antibodies level range

Overall, it is observed that the antibody index showed a decrease in the comparison between days +40 vs +110. However, this antibody index in this last sample collection is still significantly higher when comparing days 0 vs +110 (all $P < 0.0001$) for both participants without (Fig. 2A and 2B) and those with (Fig. 2C and 2D) immunity before vaccination.

4. Discussion

The seroconversion rate of 97% for the anti-S1 IgG observed in HCWs is important data and corroborates the results of phase I/II trials of CoronaVac vaccine (Zhang et al., 2021a). However, it should be noted that the necessary antibody titers for protection are not entirely known. Furthermore, in the clinical trials carried out previously to vaccine registration, the primary outcome was disease severity, so it cannot be affirmed so far whether seroconversion or antibody titers are associated with protection from infection.

Several mutations in the RBD region of the S1 protein have been shown, giving rise to the viral variants of concern, as previously described: gamma (P.1), zeta (P.2), beta (B.1.351), alpha (B.1.1.7), and B.1.325 (Claro et al., 2021, Sabino et al., 2021, Tegally et al., 2021). Such mutations confer the potential for the virus to escape the humoral immune response produced due to the disease or to viral vectors or mRNA vaccines (Garcia-beltran et al., 2021). Thus, studies

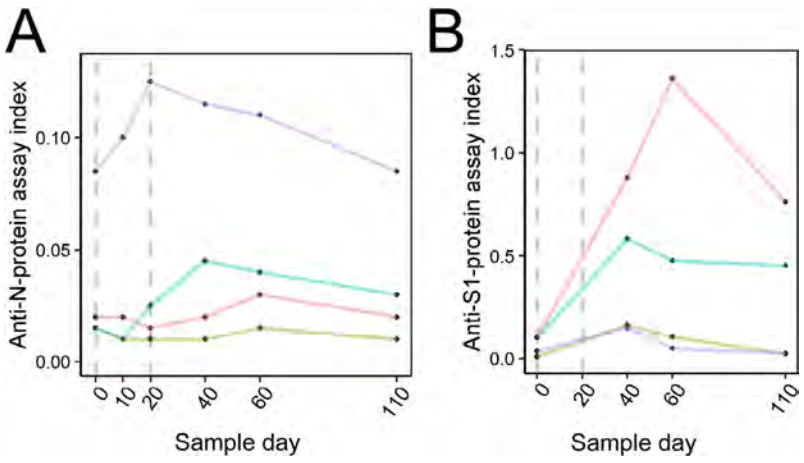


Fig. 4. Antibody rates for participants without seroconversion on day +40. Purple and green lines represent the participants with Rituximab treatment. The dotted line represents the days of the vaccine application (2 doses). (A) N-protein IgG evaluation. (B) S1-protein IgG evaluation (color version of figure is available online).

that evaluate vaccine efficacy against these new strains are valuable (Madhi et al., 2021).

Seroconversion rates observed for anti-N protein IgG could be valuable with the emergence of SARS-CoV-2 variants, considering the lower mutation levels in this protein (Dutta et al., 2020), compared to the high mutation levels in the S1 protein (Fergie and Srivastava, 2021). Thus, seroconversion of N-protein antibodies may be an alternative for the vaccine industry to produce efficient vaccines for circulating strains, including those that may arise in the future. However, more studies are needed to understand the impact of antibodies against other viral proteins in the protection against infection.

In this study, there was no difference in the analysis for the anti-N protein IgG in the reactive group, possibly due to the antibody levels present at day 0 in this group; the vaccine has not interfered in the humoral response; the group remained at the same average index. A total of 5.98% of the participants without seroconversion reported they had been previously infected by SARS-CoV-2. All of them presented seroconversion after the complete vaccination. Moreover, whether the person had experienced the disease or not, the levels of antibodies at day +40 post-vaccine were the same. This finding agrees with Krammer et al., 2021 in a study of individuals with and without previous COVID-19, given the mRNA vaccine. This same response level implies the same antigen concentration, showing no difference in individual antibody response regardless of the previous infection.

Higher index of anti-S1 antibodies were observed in comparison to the response of anti-N antibodies, corroborating what was exposed by Jiang et al., 2020. The Khoury et al., 2021 determination can be used to estimate the level of neutralizing antibodies; for a 50% protection caused by neutralizing antibodies, approximately 20% of the antibody levels observed in the ELISA assays correspond to this level of protection. And for 50% protection in severe cases, only 3% of antibody levels observed in ELISA assays correspond to such protection in severe cases (Khoury et al., 2021). Therefore, it is possible to estimate the index of neutralizing antibodies in this study.

In participants with immunosuppressive treatment (n = 2), the absence of the antibody response was probably due to rituximab having been administered approximately 1 month before the vaccine. In this situation, as described by Kado et al., 2016, there is a significant B lymphocytes decrease. Consequently, there is no production of antibodies until the B lymphocytes recover in 6 to 24 months. In such cases, the response must be evaluated after the repletion time, and re-vaccination considered with medical and clinical endorsement. Two other participants did not seroconvert on day +40. One of these had late-response seroconversion on day +60. No explanation was found for the other case, and more studies are needed to understand what interfered with the immune response.

As with the humoral response developed by other inactivated virus vaccines (Gresset-Bourgeois et al., 2017) and other vaccines for SARS-CoV-2 (Bayart et al., 2021), the dynamics of antibodies produced by CoronaVac in this study shows a peak in the antibody index followed by a sharp drop in that index. It is expected that even with these lower levels, memory B lymphocytes persist for a faster humoral response in cases of reinfection, resulting in less viral activity and minor damage to the host (Kurosaki et al., 2015). This lowest observed index has not yet been evaluated to verify whether the remaining humoral response is likely to generate a protective response against an infection.

The antibodies anti-SARS-CoV-2 produced by vaccine induction showed a significant decrease in the period of 3 to 6 months in other studies (Bayart et al., 2021, Yigit et al., 2021), as well as in this one, the need for a dose boosting has been recommended. Previous reports have already shown that the heterologous or homologous booster dose for SARS-CoV-2 vaccines (Ho et al., 2021), including CoronaVac (Keskin et al., 2021), have a surprising effect in the short term, even increasing the rate of effectiveness against the variants of concern (Yue et al., 2021). However, the antibody concentration needed to determine humoral protection remains unknown. However, it has been observed that about 6 months after completing the vaccination schedule, vaccinated individuals begin to show susceptibility to SARS-CoV-2 infection.

The immune response developed by vaccination depends not just on antibodies but primarily on neutralizing antibodies (Kurosaki et al., 2015). Both natural infection and vaccination act on the immune system in complex ways, stimulating the production of nonneutralizing antibodies (with their specific actions) and TCD4+ and TCD8+ T cells, which also act to protect against COVID-19, as shown by Tarke et al., 2021. That study evaluated the immune response to the SARS-CoV-2 variants and showed that cellular immunity-unlike the humoral response, is little affected by the virus variants. In addition to the specific immune response, innate immunity is another essential protection mechanism against infections (Kurosaki et al., 2015).

The present study has some limitations: the humoral immunity was studied semi-quantitatively, there was no quantification and titration of anti-SARS-CoV-2 antibodies, and no testing for neutralizing antibodies. The total number of participants was small, and immunosuppressed comorbidities were low in number and had diverse etiologies. More studies are needed to elucidate the vaccine response in these specific groups. However, this is the first study to evaluate the dynamics of IgG anti-N and anti-S1 production after CoronaVac immunization in the community.

The results of seroconversion have shown the importance of 2 doses for this vaccine as, until the second dose was applied, there was no change in the production of N-protein IgG, as previously described by Zhang et al., 2021 in phase I/II tests for this vaccine, with the antibody response detectable just 14 days after the second dose. The second dose is important for several types of vaccines, including mRNA vaccines, as described by Dörschug et al., 2021, resulting in a significant increase in antibody levels. Therefore, with SARS-CoV-2, there would be no difference at this point.

In conclusion, significant antibody production was observed 40 days after the first CoronaVac dose in the large majority of study participants, independent of comorbidities. The anti-N protein and anti-S1 protein antibody responses of participants without prior SARS-CoV-2 infection were comparable with those of the previously infected group, in which the immune response was maintained or optimized, with no decrease in levels.

Acknowledgments

We would like to thank all participants who agreed to participate in this study, those involved in the collection and storage of samples, and the Immunochemistry Laboratory section of Complexo Hospital de Clínicas, UFPR, and CAPES.

Funding

This work was supported by the PROPLAN/Federal University of Paraná, Curitiba-Paraná, Brazil; FINEP, Funder of Studies and Projects, Ministry of Science, Technology and Innovation, Brazil Institutional Network, Project: Laboratories for Diagnostic Tests for COVID-19 (grant number 0494/20).

Declaration of competing interest

The authors declare that there is no conflict.

Authors' contributions

LBB: data collection, data analysis and interpretation, drafting the article, final approval. SMA: data analysis and interpretation, drafting the article, critical revision, final approval. SMR: data analysis and interpretation, drafting the article, critical revision, final approval.

DA: data analysis and interpretation, drafting the article, critical revision, final approval. LLMA: data collection, drafting the article, final approval. SC: data collection, final approval. MBN: conception, data analysis and interpretation, drafting the article, critical revision, final approval, funding acquisition.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2021.115597.

References

Angeli F, Spanevello A, Reboldi G, Visca D, Verdecchia P. SARS-CoV-2 vaccines: lights and shadows. Eur J Intern Med 2021;88(March):1–8. doi: 10.1016/j.ejim.2021.04.019. Available from.

Barchuk A, Shirokov D, Sergeeva M, Tursun-zade R, Dudkina O, Tychkova V, et al. Evaluation of the performance of SARS-CoV--2 antibody assays for the longitudinal population-based study of COVID-19 spread in St. Petersburg, Russia. J Med Virol 2021;93:5846–52.

Bayart J, Douxfils J, Gillot C, David C, Mullier F, Elsen M, et al. Waning of IgG, total and neutralizing antibodies 6 months post-vaccination with BNT162b2 in healthcare workers. Vaccines 2021;9:1092. doi: 10.3390/vaccines9101092.

Brasil, Ministério da Saúde, COVID-19 Vacinação doses Aplicadas. [cited 2021 Jun 24] Available from: https://viz.saude.gov.br/extensions/DEMAS_C19Vacina/DEMAS_C19Vacina.html

Claro IM, da Silva Sales FC, Ramundo MS, Candido DS, Silva CAM, de Jesus JG, et al. Local transmission of SARS-CoV-2 lineage B.1.1.7, Brazil, December 2020. Emerg Infect Dis 2021;27(3):970–2.

Dörschug A, Frickmann H, Schwanbeck J, Yilmaz E, Mese K, Hahn A, et al. Comparative assessment of sera from individuals after S-Gene RNA-based SARS-CoV-2 vaccination with spike-protein-based and nucleocapsid-based serological assays. Diagnostics 2021;11(3):426.

Dutta NK, Mazumdar K, Gordy JT. The nucleocapsid protein of SARS–CoV-2: a target for vaccine development. J Virol 2020;94(13):1–2.

Fergie J, Srivastava A. Immunity to SARS-CoV-2: lessons learned. Front Immunol 2021;12(March):1–12.

Garcia-beltran WF, Lam EC, Denis KS, Nitido AD, Garcia ZH, Hauser BM, et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. Cell 2021;184:2372–83.

Golob JL, Lugogo N, Lauring AS, Lok AS. SARS-CoV-2 vaccines: a triumph of science and collaboration. JCI Insight 2021;6:1–11.

Gresset-Bourgeois V, Leventhal PS, Pepin S, Hollingsworth R, Kazek-Duret M-P, De Bruijn I, et al. Quadrivalent inactivated influenza vaccine (VaxigripTetra™). Expert Rev Vaccines 20171–11. doi: 10.1080/14760584.2018.1407650.

Ho T, Chen Y, Chan H, Chang C, Chuang K, Lee C, et al. the effects of heterologous immunization with prime-boost COVID-19 vaccination against SARS-CoV-2. Vaccines 2021;9:1163. doi: 10.3390/vaccines9101163.

Jiang H, Li Y, Zhang H, Wang W, Yang X, Qi H, et al. SARS-CoV-2 proteome microarray for global profiling of COVID-19 specific IgG and IgM responses. Nat Commun 2020;11(1):1–11.

Kado R, Sanders G, Joseph, McCune W. Suppression of normal immune responses after treatment with rituximab. Curr Opin Rheumatol 2016;28(3):251–8.

Keskin AU, Bolukcu S, Ciragil P, Topkaya AE. SARS-CoV-2 specific antibody responses after third CoronaVac or BNT162b2 vaccine following two-dose CoronaVac vaccine regimen. J Med Virol 2021. doi: 10.1002/jmv.27350.

Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med 2021;27:1205–11.

Krammer F, Srivastava K, Alshammary H, Amoako AA, Awawda MH, Beach KF, et al. Antibody responses in seropositive persons after a single dose of SARS-CoV-2 mRNA vaccine. N Engl J Med 2021;384(14):1372–4.

Kumar SU, Priya NM, Priyanka SRN, Nikita K, Thirumal JD. A review of novel coronavirus disease (COVID - 19): based on genomic structure, phylogeny, current shreds of evidence, candidate vaccines, and drug repurposing. 3 Biotech 2021;11:198. doi: 10.1007/s13205-021-02749-0.

Kurosaki T, Kometani K, Ise W. Memory B cells. Nat Rev Immunol 2015;15:149–59. doi: 10.1038/nri3802.

Madhi SA, Baillie V, Cutland CL, Voysey M, Koen AL, Fairlie L, et al. Efficacy of the ChAdOx1 nCoV-19 covid-19 vaccine against the B.1.351 variant. N Engl J Med 2021;384(20):1885–98.

Murray MJ, McIntosh M, Atkinson C, Mahungu T, Wright E, Chatterton W, et al. Validation of a commercially available indirect assay for SARS-CoV-2 neutralising antibodies using a pseudotyped virus assay. J Infect 2021;82(5):170–7.

Padoan A, Bonfante F, Cosma C, Di Chiara C, Sciacovelli L, Pagliari M, et al. Analytical and clinical performances of a SARS-CoV-2 S-RBD IgG assay: comparison with neutralization titers. Clin Chem Lab Med 2021;59:1444–52.

Palacios R, Batista AP, Albuquerque CSN, Patiño EG, Santos J do P, Tilli Reis Pessoa Conde M, et al. Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in brazil: the PROFISCOV study. SSRN Electron J 2021;21: 853. doi: 10.1186/s13063-020-04775-4.

Papenburg J, Cheng MP, Corsini R, Caya C, Mendoza E, Manguiat K, et al. Evaluation of a commercial culture-free neutralization antibody detection kit for severe acute respiratory syndrome-related coronavirus-2 and comparison with an antireceptor-binding domain enzyme-linked immunosorbent assay. Open Forum Infect Dis 2021;8(6) ofab220. doi: 10.1093/ofid/ofab220.

Sabino EC, Buss LF, Carvalho MPS, Prete CA, Crispim MAE, Fraiji NA, et al. Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. Lancet 2021;397(10273):452–5.

Saelens X, Schepens B. Single-domain antibodies make a difference. Science (80-) 2021;371(6530):681–2.

Tarke A, Sidney J, Methot N, Yu ED, Zhang Y, Dan JM, Goodwin B, et al. Impact of SARS-CoV-2 variants on the total CD4 $^{+}$ and CD8 $^{+}$ T cell reactivity in infected or vaccinated individuals. Cell Rep Med 2021;2: 100355. doi: 10.1016/j.xcrm.2021.100355.

Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. Nature 2021;592:438–43.

World Health Organization (WHO), Coronavirus disease (COVID-19) advice for the public. [cited 2021 Jun 24]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public

World Health Organization (WHO), WHO coronavirus (COVID-19) dashboard. [cited 2021 Jul 5]. Available from: https://covid19.who.int/

Yigit M, Ozkaya-Parlakay A, Cosgun Y, Ince YE, Bulut YE, Senel E. Should a third booster dose be scheduled after two doses of CoronaVac? A single-center experience. J Med Virol 20211–4. doi: 10.1002/jmv.27318.

Yue L, Zhou J, Zhou Y, Yang X, Xie T, Yang M, et al. Antibody response elicited by a third boost dose of inactivated SARS-CoV-2 vaccine can neutralize SARS-CoV-2 variants of concern. Emerg Microbes Infect 2021. doi: 10.1080/22221751.2021.1996210.

Zeng W, Liu G, Ma H, Zhao D, Yang Y, Liu M, et al. Biochemical characterization of SARS-CoV-2 nucleocapsid protein. Biochem Biophys Res Commun 2020;527(3):618–23.

Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021a;21(2):181–92.

Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis [Internet] 2021b;21(2):181–92. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1473309920308434.

1.10. CoronaVac induz memória imunológica eficiente e semelhante à de pacientes convalescentes, mostra estudo chinês

Uma pesquisa publicada na revista Clinical Microbiology and Infection demonstrou que a CoronaVac, vacina do Butantan e da Sinovac, apresenta alta eficácia na resposta humoral (produção de anticorpos) e na resposta celular (células T CD4+ e CD8+) contra o SARS-CoV-2, e promove memória imunológica comparável à de pacientes convalescentes. O estudo foi conduzido por pesquisadores chineses da Universidade de Nanjing entre janeiro e fevereiro de 2021.

Os cientistas analisaram o perfil de resposta imune de 100 profissionais da saúde (37 homens e 63 mulheres) com idades entre 23 e 59 anos que foram vacinados com a CoronaVac. Amostras de sangue foram coletadas antes da primeira dose (T1), duas semanas após a primeira dose (T2), duas semanas após a segunda dose (T3) e oito a dez semanas após a segunda dose (T4).

Todos os participantes apresentaram soroconversão (produção de anticorpos) 14 dias após a segunda dose, sendo que 98% dos indivíduos produziram anticorpos IgG específicos contra a proteína Spike e 85% tinham anticorpos capazes de neutralizar o SARS-CoV-2.

Além disso, foram detectadas respostas potentes de células T CD4+ e CD8+ de memória, com níveis comparáveis aos encontrados em pacientes recuperados que já tiveram Covid-19. Segundo os autores, células T CD4+ e CD8+ específicas para o coronavírus já foram associadas à redução da gravidade da doença.

Os participantes do estudo também apresentaram células B de memória (produtoras de anticorpos) que foram mantidas até a análise final, oito a dez semanas após a segunda dose. Essas células são responsáveis por reconhecer os

antígenos do vírus e são capazes de reagir rapidamente à infecção.

Os pesquisadores afirmam que o estudo traz novas informações sobre a imunobiologia de vacinas de vírus inativado e pode ter implicações em estratégias vacinais no futuro. "Nós identificamos células T CD4+ de memória associadas às células B de memória específicas para proteína Spike e às células T CD8+ de memória, indicando um desenvolvimento convergente da imunidade adaptativa humoral e celular", destacam.

Fatores que interferem na resposta

Metade dos participantes recebeu a segunda dose da vacina com um intervalo de 14 a 21 dias, enquanto os outros 50 receberam a segunda dose 22 a 30 dias depois da primeira. O grupo imunizado com um intervalo maior entre as doses apresentou maiores taxas de anticorpos neutralizantes e uma maior porcentagem de células B específicas para a proteína Spike e de células de memória T CD4+ e CD8+.

A idade também influenciou a resposta imune: pessoas entre 20 e 40 anos apresentaram maiores títulos médios de anticorpos neutralizantes (GMT 42) do que indivíduos com mais de 40 anos (GMT 26). Apesar disso, ambos os grupos tinham níveis semelhantes de anticorpos IgG específicos para proteína Spike.

Publicado em: 26/10/2021

ARTICLE IN PRESS

Clinical Microbiology and Infection xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine

Yuxin Chen [1, 5, †], Shengxia Yin [2, 5, †], Xin Tong [2, 5, †], Yue Tao [1], Jun Ni [1], Jie Pan [1], Ming Li [3], Yawen Wan [4], Minxin Mao [3], Yali Xiong [2], Xiaomin Yan [2], Yue Yang [2], Rui Huang [2, 5], Chao Wu [2, 5], Han Shen [1, *]

[1] Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China
[2] Department of Infectious Diseases, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China
[3] Nanjing Drum Tower Hospital Clinical College of Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China
[4] Department of Infectious Diseases, Nanjing Nanjing Drum Tower Hospital Clinical College of Xuzhou Medical University, China
[5] Institute of Viruses and Infectious Diseases, Nanjing University, Jiangsu, China

ARTICLE INFO

Article history:
Received 3 July 2021
Received in revised form
6 October 2021
Accepted 16 October 2021
Available online xxx

Editor: L. Kaiser

Keywords:
Adaptive responses
B cells
COVID-19
Inactivated virus vaccine
Neutralization
T cells

ABSTRACT

Objective: The dynamic adaptive immune responses elicited by the inactivated virus vaccine CoronaVac remain elusive.

Methods: In a prospective cohort of 100 healthcare professionals naïve for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) who received two doses of CoronaVac, we analysed SARS-CoV-2-specific humoral and cellular responses at four different timepoints, including before vaccination (T1), 2 weeks after the first dose (T2), 2 weeks after the booster dose (T3), and 8–10 weeks after the booster dose (T4). SARS-CoV-2-specific antibodies, serum neutralizing activities, peripheral B cells, $CD4^+$ and $CD8^+$ T cells and their memory subsets were simultaneously measured in this cohort.

Results: SARS-CoV-2 spike-specific IgG responses reached a peak (geometric mean titre (GMT) 54827, 30969–97065) after two doses and rapidly declined (GMT 502, 212–1190) at T4, whereas suboptimal IgA responses were detected (GMT 5, 2–9). Spike-specific circulating B cells (0.60%, 0.46–0.73% of total B cells) and memory B cells (1.18%, 0.92–1.44% of total memory B cells) were effectively induced at T3 and sustained over time (0.33%, 0.23–0.43%; 0.87%, 0.05–1.67%, respectively). SARS-CoV-2-specific circulating $CD4^+$ T cells (0.57%, 0.47–0.66%) and $CD8^+$ T cells (1.29%, 1.04–1.54%) were detected at T3. At T4, 0.78% (0.43–1.20%) of memory CD4+ T cells and 0.68% (0.29–1.30%) of memory CD8+ T cells were identified as SARS-CoV-2-specific, while 0.62% (0.51–0.75%) of $CD4^+$ T cells and 0.47% (0.38–0.58%) of $CD8^+$ T cells were SARS-CoV-2-specific terminally differentiated effector memory cells. Furthermore, age and interval between doses affected the magnitude of CoronaVac-induced immune responses. SARS-CoV-2 memory $CD4^+$ T cells were strongly associated with both receptor binding domain (RBD)-specific memory B cells (r 0.87, p <0.0001) and SARS-CoV-2-specific memory $CD8^+$ T cells (r 0.48, p <0.0001).

Conclusions: CoronaVac induced robust circulating and memory B cell and T cell responses. Our study offers new insight into the underlying immunobiology of inactivated virus vaccines in humans and may have implications for vaccine strategies in the future. **Yuxin Chen, Clin Microbiol Infect 2021;■:1**

Introduction

Vaccines are the cornerstone of the management of infectious disease outbreaks and the surest means to defuse pandemic risk. CoronaVac (Sinovac Biotech, China), a whole-virion chemically inactivated vaccine against severe acute respiratory syndrome

* Corresponding author: Han Shen, Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China.
E-mail address: shenhan10366@sina.com (H. Shen).
† Yuxin Chen, Shengxia Yin and Xin Tong contributed equally.

https://doi.org/10.1016/j.cmi.2021.10.006
1198-743X/

Please cite this article as: Chen Y et al., Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.10.006

coronavirus 2 (SARS-CoV-2), has so far been inoculated into at least 243 million individuals from more than 45 countries. A large, observational study in Chile indicated that two doses of CoronaVac had a vaccine effectiveness of 65.9% against coronavirus disease 2019 (COVID-19), 90.3% against intensive care unit admission and 86.3% against COVID-19-related death [1]. Nevertheless, few studies on CoronaVac recipients focused largely on binding and/or neutralizing antibodies (NAbs) as primary endpoints, while vaccine-induced cellular immune responses remain elusive.

It is well established that three fundamental components of the adaptive immune system (B cells, $CD4^+$ and $CD8^+$ T cells) are essential to control SARS-CoV-2 infection [2–7]. Despite the immune correlates of protection remaining unknown [8,9], antibodies and T-cell responses are important for the resolution of primary SARS-CoV-2 infection. Additionally, SARS-CoV-2 infection induced various immunological memory components displaying distinct kinetics [10].

Recently, we conducted a prospective, observational cohort study (NCT04729374) with 100 healthcare personnel in a tertiary hospital in Nanjing, China. Most sera elicited by two-dose CoronaVac were capable of effectively neutralize the ancestral strain, Alpha and Epsilon variants, but not Beta and Gamma variants bearing E484K mutation [11]. In this current study, we provided data from this cohort with new insights into the kinetics of vaccine-induced humoral and cellular immune responses, including circulating antibodies, antigen-specific B cells, $CD4^+$ and $CD8^+$ T cells, as well as their memory subsets at four timepoints extending up to 8–10 weeks post two-dose immunization. The impact of gender, age and interval between doses on the magnitude of vaccine responses were further analysed. The interrelationships between antibody and cellular responses were also evaluated.

Materials and methods

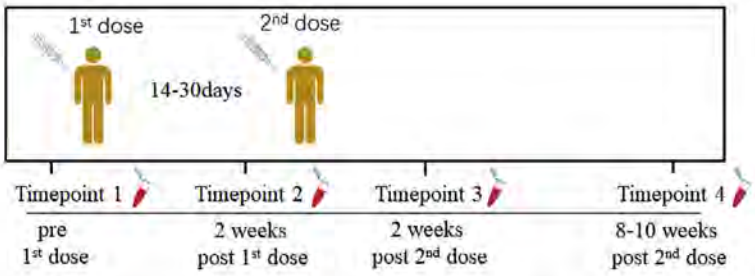
Study cohort and sample collection

A total of 100 healthcare professionals were enrolled in a prospective study (NCT04729374) from January to February 2021 in Nanjing Drum Tower Hospital. All participants tested negative for SARS-CoV-2 infection at screening and provided written informed consent. The clinical trial protocol was approved by the hospital ethics committee (2021-034-01). Two cohorts of COVID-19 convalescent patients were included, and their demographic characteristics are provided in Fig. 1. In the first cohort, serum samples were collected from 26 convalescent patients on a 4-week follow-up visit after hospital discharge, while peripheral blood mononuclear cells (PBMCs) from 12 convalescent patients were collected 16 months after COVID-19 infection in the second cohort.

SARS-CoV-2-specific humoral and cellular responses

The quantification of antigen-specific antibodies against SARS-CoV-2 and serum neutralization activities were performed as previously described [11,12]. Fluorescence-labelled ectodomain of the spike or receptor binding domain (RBD) proteins were used as probes to identify SARS-CoV-2-specific B cells and memory B cells. PBMCs were stimulated with SARS-CoV-2 peptide pools to measure antigen-specific $CD4^+$ and $CD8^+$ T cells. The details of peptide pools,

A



B

variables(n[%] or median[IQR]	COVID-19 vaccinee cohort (n=100)	convalescent COVID-19 patient cohort 1 (n=26)	convalescent COVID-19 patient cohort 2 (n=12)
Sex			
Male	37 (37%)	14 (53.8%)	6 (50%)
Female	63 (63%)	12 (46.2%)	6 (50%)
Age (yr)	35 (28, 41)	47 (34, 58)	50 (28, 59)
Age group, years			
18-30	35 (36%)	3 (11.5%)	2 (16.7%)
31-40	39 (38%)	4 (15.4%)	2 (16.7%)
41-50	16 (16%)	7 (26.9%)	2 (16.7%)
51-60	10 (10%)	4 (15.4%)	6 (50%)
Sample types	serum and PBMC	serum	PBMC
Sample collection data	Jan-May 2021	March-April 2020	May-Jun 2021
Disease onset time	NA	Jan-Feb 2020	Jan-Feb 2020
Disease severity			
Severe	NA	7 (26.9%)	2 (16.7%)
Non-severe	NA	19 (73.1%)	10 (83.3%)
Co-morbidities	2 (2%)	4 (15.4%)	0 (0%)

Fig. 1. The study design and the characteristics of participants in our cohort. (A) The study design of our vaccine cohort. (B) The characteristics of three study cohorts used in our study, including the vaccine cohort who received two doses of CoronaVac, the convalescent coronavirus disease 2019 (COVID-19) patient cohort 1 and the convalescent COVID-19 patient cohort 2.

Please cite this article as: Chen Y et al., Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.10.006

conjugation of antibodies, sample staining and statistical analysis were presented in the Supplementary Material.

Results

Study design

One hundred healthcare workers were enrolled in this study; their ages ranged from 23 to 59 years (median 35), and 63 (63%) were female (Fig. 1). All participants finished two doses of CoronaVac; 50 first-dose recipients received the second dose within 14–21 days after the first dose, and 50 received the second dose between 22 and 30 days. To investigate the kinetics of the immune responses following both primary and secondary immunizations, the serum and PBMC samples were collected for immunological analysis at four different timepoints: pre-vaccine baseline (T1), 2 weeks following the first dose (T2), 2 weeks following the second dose (T3), and 8–10 weeks following the second dose (T4).

SARS-CoV-2-specific humoral responses

At baseline, all participants had undetectable levels of IgM, IgG and IgA antibodies specific for the ectodomain of the spike protein (Spike), nucleocapsid protein (NP) and RBD protein (Fig. 2A–F and Supplementary Material Fig. S1). Two doses of CoronaVac significantly boosted antibody responses achieving the peak level of humoral immunity, and 100% of the participants seroconverted after two doses of immunization. Specifically, 98 vaccinees (98%) were anti-spike IgG-positive (geometric mean titre (GMT) 54827, 30969–97065) and 23 (23%) were IgA-positive (GMT 5, 2–9); 85% (85/100) and 29% (29/100) of sera at T3 were able to neutralize the ancestral strain and B.1.617.1, respectively. The B.1.617.1 variant was 2.96-fold resistant to neutralization by sera from CoronaVac recipients, compared to the ancestral strain (Fig. 2G). At T4, spike-specific and NP-specific IgG responses declined significantly, and vaccinee sera had a significantly higher anti-spike IgG titre but remarkable lower IgA responses compared to those in convalescent sera (Fig. 2A–F).

SARS-CoV-2-specific B-cell responses

The first dose of CoronaVac induced a significant proportion of spike-specific B cells (0.32%, 0.27–0.38%), which expanded after the second dose (0.60%, 0.46–0.73%) despite no statistical differences, and slightly reduced at T4 (0.33%, 0.23–0.43%) (Fig. 3A). Similarly, the frequency of spike-specific memory B cells at T3 was on average 1.18% (0.92–1.44%) and gradually reduced to 0.87% (0.10–1.63%) at T4. A similar pattern was observed for RBD-specific B cells and memory B cells (Fig. 3B). RBD-specific B cells at T4 correlated with serum titres that achieved 50% pseudovirus neutralization (pNT50) against the D614G variant, B.1.1.7 and B.1.526 (Fig. 3C). Vaccinees displayed comparable magnitudes of spike-specific B cells as well as RBD-specific memory B cells, but lower levels of spike-specific memory B cells and RBD-specific B cells at the T4 timepoint, compared to COVID-19-recovered donors (Fig. 3A,B).

Immunoglobin (Ig) isotypes among the antigen-specific memory B-cell population shifted with time (Fig. 3A,B). After primary immunization, ~23% of RBD-specific memory B cells were IgG^+ and ~22% were IgM^+. The frequency of IgG^+ memory B cells surged to ~45% following the second dose, and slightly increased to ~55% 8–10 weeks after full vaccination. RBD-specific IgA^+ memory B-cell frequency was ~13% at both T2 and T3 and slightly increased to ~22% at T4.

B-cell analyses were extended to *in vitro* stimulation of memory B cells which differentiate into antibody-secreting cells (ASCs) by ELISPOT assay among a small portion of participants (Fig. 3D). The first dose induced positive spike-specific and RBD-specific B cells in 38.9% (21/54) and 22.2% (12/54) of subjects, respectively. The second dose further boosted spike-specific and RBD-specific antibody-secreting B cells in 57.7% (15/26) and 57.7% (15/26) of subjects, respectively. The frequency of spike-specific and RBD-specific memory B cells was stable at T4, and were detected in 70.2% (33/47) and 87.2% (41/47) of subjects.

The magnitude of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T-cell responses

SARS-CoV-2-specific T-cell responses were analysed by *ex vivo* stimulation with SARS-CoV-2 peptide pools covering the most commonly recognized T-cell epitopes [4], including S, M, E, N, ORF3a and ORF7/8 (Supplementary Material Fig. S4). Robust expanded SARS-CoV-2-specific $CD4^+$ T cells were detectable in 61.5% (48/78), 74.2% (69/93) and 75.0% (60/80) of the subjects at T2, T3 and T4, respectively (Fig. 4A). SARS-CoV-2-specific $CD4^+$ T-cell responses were also significantly elevated after the primary immunization (0.57%, 0.47–0.66%) compared to that at T1 (0.08%, 0.02–0.27%). The specific $CD4^+$ T cells (0.83%, 0.67–1.00%) elicited after two doses remained stable at T4 (1.22%, 0.96–1.48%).

Minimal circulating SARS-CoV-2 $CD8^+$ T-cell responses (0.06%, 0.05–0.07%) were detected at T1 baseline (Fig. 4B); 80% (52/65) of participants had detectable SARS-CoV-2 $CD8^+$ T-cell responses (0.69%, 0.54–0.84%) at T2. The boosting immunization induced 83.9% (78/93) of subjects with positive SARS-CoV-2 $CD8^+$ T-cell responses (1.29%, 1.04–1.54%), which steadily increased to 1.61% (1.21–2.02%) at T4. Spike-specific $CD4^+$ or $CD8^+$ T cells displayed a similar kinetic to the SARS-CoV-2-specific $CD4^+$ or $CD8^+$ T cells. Interestingly, CoronaVac also induced $CD4^+$ and $CD8^+$ T-cell responses specific to HCoV-OC43 and HCoV-299E spike glycoprotein (Supplementary Material Fig. S5).

At T4, 0.78% (0.43–1.20%) of memory $CD4^+$ T cells and 0.68% (0.29–1.30%) of memory $CD8^+$ T cells were identified as SARS-CoV-2-specific (Fig. 4C). Vaccinees had similar magnitudes of SARS-CoV-2-specific memory $CD4^+$ T cells, $CD8^+$ T cells and spike-specific memory $CD4^+$ T cells, but a lower level of spike-specific memory $CD8^+$ T cells, compared to convalescent donors. The majority of virus-specific $CD8^+$ T cells were identified as CD45RA CCR7 effector memory (T_{EM}) or $CD45RA^+CCR7$ terminally differentiated effector (T_{EMRA}) [13,14]. Among vaccinees at T4, 0.62% (0.51–0.75%) and 0.43% (0.30–0.57%) of $CD4^+$ T cells were SARS-CoV-2-specific T_{EMRA} and T_{EM}, respectively (Fig. 4D), whereas 0.48% (0.38–0.58%) and 0.79% (0.66–0.92%) of $CD8^+$ T cells were SARS-CoV-2-specific T_{EMRA} and T_{EM}, respectively (Fig. 4E). Convalescent patients displayed a similar proportion of virus-specific T_{EMRA} and T_{EM} as the vaccinees. Our data suggest that CoronaVac effectively induced virus-specific memory $CD4^+$ T cells and $CD8^+$ T cells as well as effector populations.

Factors associated with adaptive responses to SARS-CoV-2 inactivated virus vaccine

There were no relationships identified between gender and the magnitude of SARS-CoV-2-specific adaptive responses (Fig. 5A). Consistent with a previous report [15], the participants between 20 and 40 years old had significantly higher neutralizing titres (GMT 42, 33–52) against the ancestral strain, compared to the participants between 40 and 60 years old (GMT 26, 19–37) (Fig. 5B and Supplementary Material Fig. S6A). Despite the fact that young participants had a higher magnitude of serum neutralizing activities than elder individuals, both groups had a comparable level of anti-spike IgG, suggesting potential qualitative differences in spike-

Please cite this article as: Chen Y et al., Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.10.006

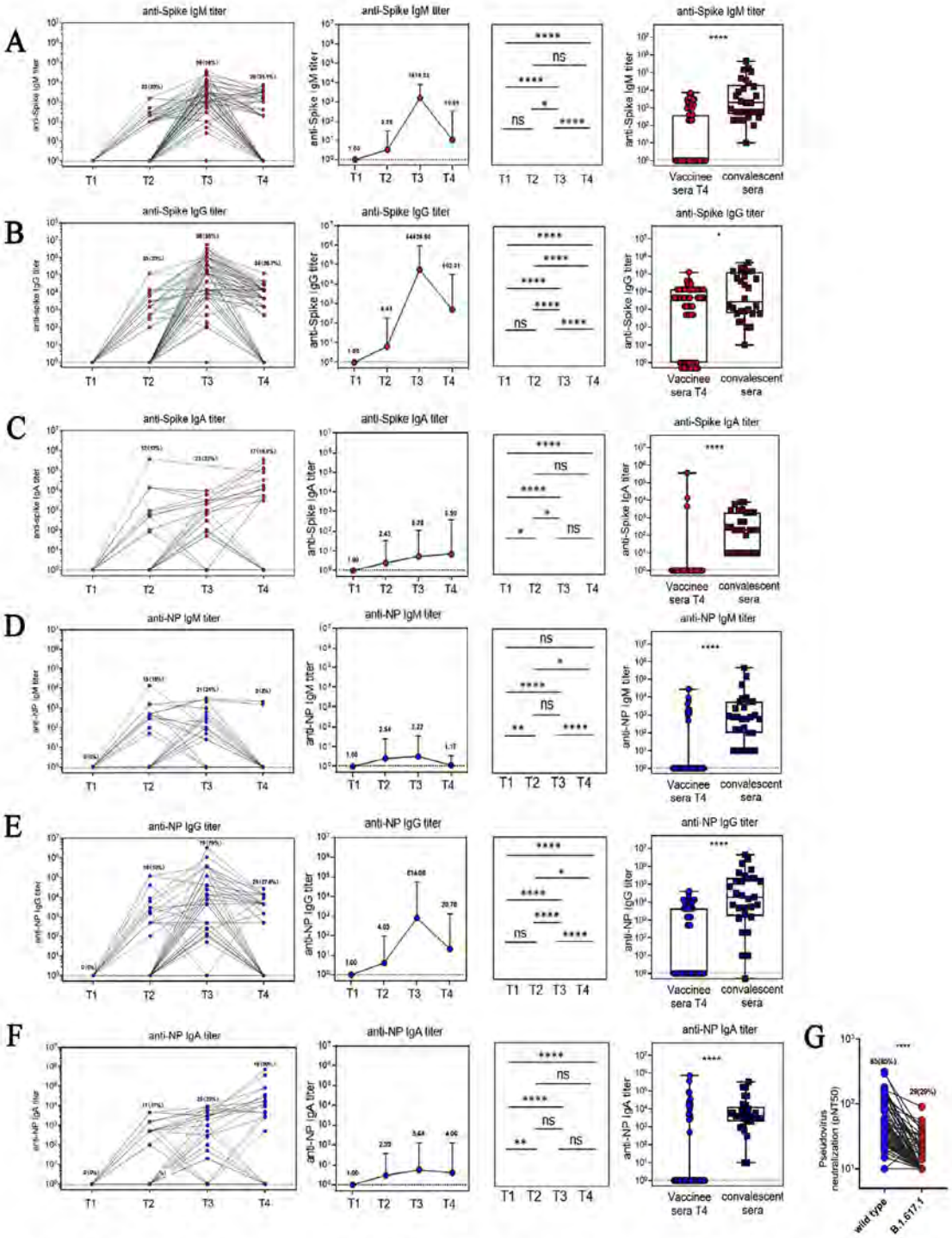


Fig. 2. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific antibody responses following CoronaVac immunization. (A–F) Dynamic antibody titres for (A) anti-spike IgM, (B) anti-spike IgG, (C) anti-spike IgA, (D) anti-NP IgM, (E) anti-NP IgG, and (F) anti-NP IgA at four time points were analysed, including baseline (T1), 2 weeks after the first dose of CoronaVac (T2), 2 weeks post the booster dose (T3), and 8–10 weeks after the booster dose (T4). In addition, the antigen-specific titres were also compared between sera collected from vaccinees at T4 timepoints and convalescent patient cohort 1 (8–10 weeks post symptom onset). Dotted lines indicate the limit of detection (LOD) for the assay. Statistics were calculated using Wilcoxon matched-pairs signed rank for comparison between timepoints and unpaired Wilcoxon test for comparison between vaccinees at T4 and convalescent patients from cohort 1. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns, no significant difference.

Please cite this article as: Chen Y et al., Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.10.006

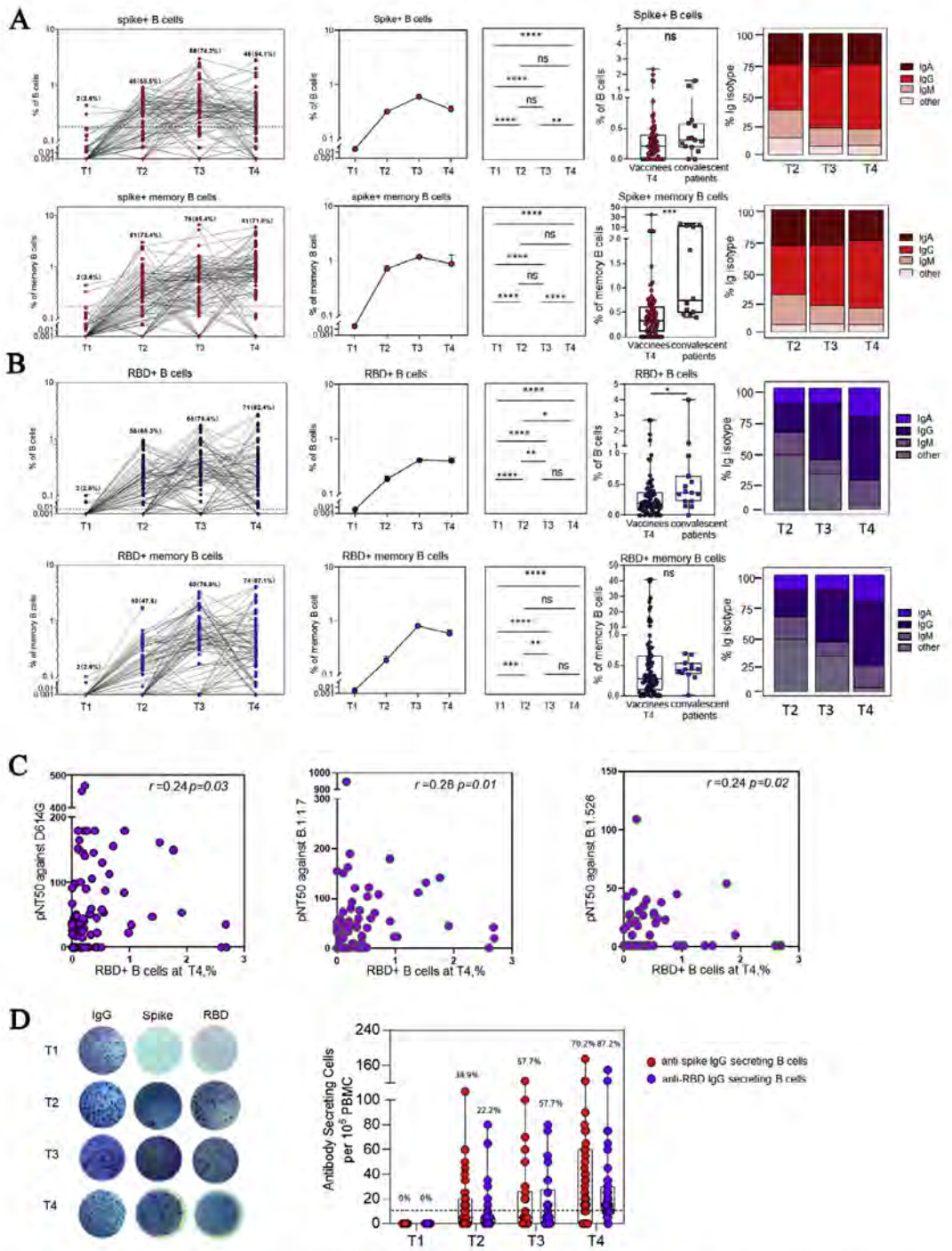


Fig. 3. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific circulating B cell and memory B cell responses following CoronaVac immunization. (A) Frequency of spike$^+$ B cells and spike$^+$ memory B cells over time in vaccinees. Frequency of spike$^+$ B cells or memory spike$^+$ B cells was compared between vaccinees at the T4 timepoint and coronavirus disease 2019 (COVID-19) convalescent patients from cohort 2. Proportion of IgG and IgM isotypes over time was determined in spike-specific circulating B cells or memory B cell compartments. (B) Frequency of RBD$^+$ B cells and RBD$^+$ memory B cells over time in vaccinees. Frequency of RBD$^+$ B cells or memory RBD$^+$ B cells were compared between vaccinees at T4 timepoint and convalescent patients from cohort 2. (C) Association analyses for the frequency of RBD-specific circulating B cells at T4 timepoint and pNT50 against D614G, B.1.1.7, and B.1.526, respectively. P <0.05 was considered to be statistically significant. Statistics were analysed using Wilcoxon matched-pairs signed rank between timepoints. (D) B cell ELSPOT assay for a representative vaccine recipient in our cohort over time (left panel). The frequency of anti-spike and anti-RBD antibody-secreting cells at different time points (right panel). * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001, ns, no significant difference.

Please cite this article as: Chen Y et al., Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.10.006

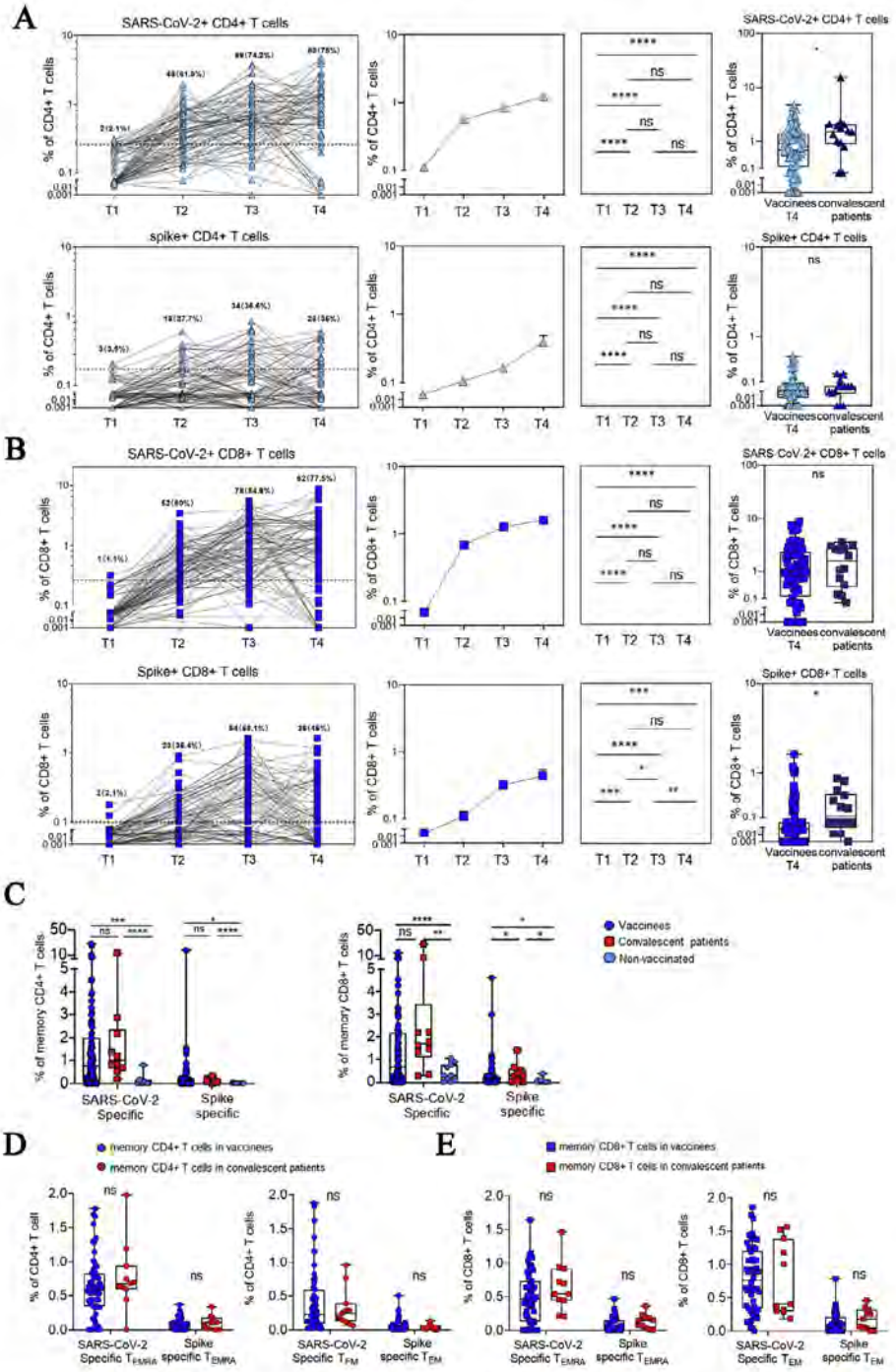


Fig. 4. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific circulating $CD4^+$ and $CD8^+$ T cell responses following CoronaVac immunization. (A) Frequency of SARS-CoV-2-specific (top) and spike-specific (bottom) $CD4^+$ T cells over time among vaccinees, the magnitude of which at T4 were further compared with that in convalescent patients from cohort 2. (B) Frequency of SARS-CoV-2-specific (top) and spike-specific (bottom) $CD8^+$ T cells over time in vaccinees, the magnitudes of which at T4 were further compared with convalescent patients from cohort 2. (C) Proportion of SARS-CoV-2-specific (left) and spike-specific (right) memory $CD4^+$ and memory $CD8^+$ T cells among vaccinees at T4 timepoint, convalescent patients in cohort 2 and non-vaccinated healthy subjects. (D-E) Distribution of terminally differentiated effector memory T cells (TEMRA) and effector memory T cells (TEM) in $CD4^+$ T cells (D) and $CD8^+$ T cells (E) from vaccinees at T4 timepoint and convalescent subjects from cohort 2. Statistics were analysed using Wilcoxon matched-pairs signed rank test between timepoints. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns, no significant difference.

Please cite this article as: Chen Y et al., Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.10.006

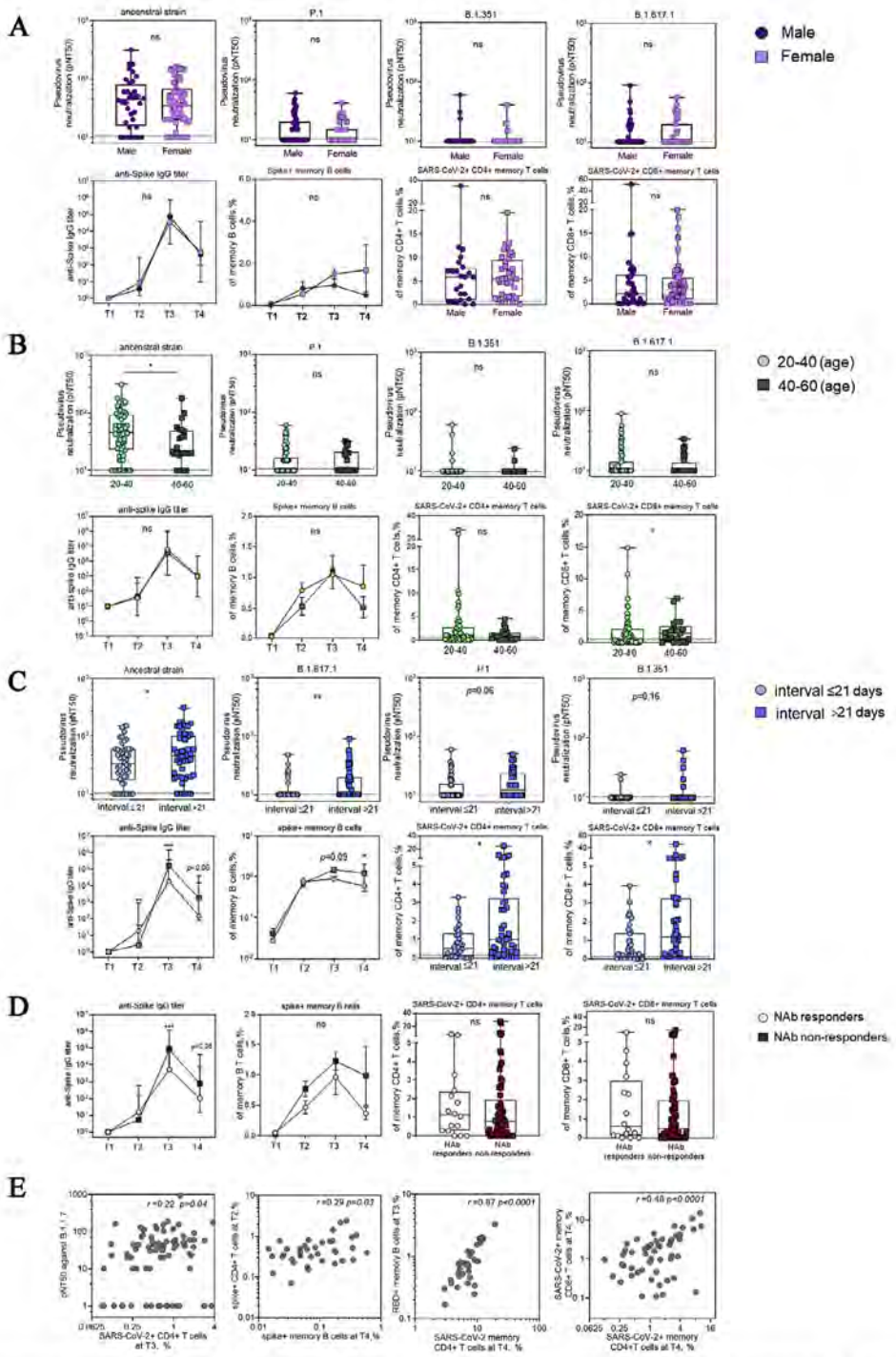


Fig. 5. Association of various factors with vaccine-elicited adaptive responses. (A-C) Serum titres that achieved 50% pseudovirus neutralization (pNT50) against the ancestral strain, the P.1, the B.1.351, the B.1.617.1, anti-spike IgG titre, the frequency of spike-specific memory B cells, the frequency of SARS-CoV-2-specific memory $CD4^+$ and $CD8^+$ T cells compared with (A) gender, (B) age, and (C) interval between doses. (D) Anti-spike IgG titre, the frequency of spike-specific memory B cells, and the frequency of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ memory T cells among neutralizing antibody (NAb) responders versus NAb non-responders. (E) Correlation analysis of pNT50 against B.1.1.7 and SARS-CoV-2-specific $CD4^+$ T cells at T3 timepoint, correlation analysis of spike-specific memory B cells at T4 and spike-specific $CD4^+$ T cells at T2, correlation analysis of RBD^+ memory B cells at T3 and SARS-CoV-2 memory $CD4^+$ T cells at T4, and correlation analysis of SARS-CoV-2-specific memory $CD8^+$ T cells at T4 and SARS-CoV-2-specifc memory $CD4^+$ T cells responses at T4. Statistics were analysed using unpaired Wilcoxon test between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns, no significant difference.

Please cite this article as: Chen Y et al., Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.10.006

specific humoral immunity. There was no association between age and vaccine-induced cellular responses, including spike-specific memory B cells, virus-specific $CD4^+$ T cells and $CD8^+$ T cells. Our data suggest potentially relevant age-related changes in neutralizing activities but not virus-specific T cell or B cell responses.

Furthermore, the interval between two doses is a critical factor that affects the magnitude of the immune responses. The participants with a dosing interval >21 days had higher neutralizing antibody (NAb) titres against the ancestral strain and B.1.617.1, compared to the group with the interval ≤21 days (Fig. 5C), which might be associated with the increased anti-spike IgG responses. The interval >21 days also induced a higher percentage of spike-specific B cells, SARS-CoV-2-specific memory $CD4^+$ T cells and $CD8^+$ T cells, compared to the group with an interval ≤21 days. Consistently, the interval correlated with spike-specific $CD4^+$ T cell responses at T3 (Supplementary Material Fig. S6B).

We also addressed the potential relationship between humoral immunity and cellular immune parameters. NAb responders had a significantly higher level of anti-spike IgG responses compared to NAb non-responders at T3 (Fig. 5D). There is a trend that NAb responders generated higher spike-specific memory B cells among total memory B cells than in NAb non-responders. Of note, NAb non-responders generated comparable levels of SARS-CoV-2-specific memory $CD4^+$ and $CD8^+$ T cells. Additionally, neutralization titres against B.1.1.7 correlated with SARS-CoV-2-specific $CD4^+$ T cells at T3 (r 0.22, p 0.04), and spike-specific memory B cells at T4 correlated with spike-specific $CD4^+$ T cells at T2 (r 0.29, p 0.03). SARS-CoV-2 memory $CD4^+$ T cells at T4 were strongly associated with both RBD-specific memory B cells at T3 (r 0.87, p <0.0001) as well as SARS-CoV-2-specific memory $CD8^+$ T cells at T4 (r 0.48, p <0.0001) (Fig. 5E).

Discussion

Here we provided an extensive characterization of adaptive immune responses specific to SARS-CoV-2 following SARS-CoV-2 inactivated vaccine. Our data are encouraging and fill the gaps in our knowledge of immune responses elicited by CoronaVac. First, we observed robust IgG responses specific to spike, RBD and NP after each dose of CoronaVac. However, these antigen-specific IgG responses decayed rapidly within 6–8 weeks, consistent with observations in COVID-19 patients and vaccinees [12,16]. Such waned antibody responses in COVID-19 patients might be caused by a lack of germinal centre (GC) reaction [17], which is essential to generate long-lived and high-affinity antibody responses. Despite the rapid decline in IgG responses, vaccinees displayed higher spike-specific IgG responses but lower RBD-specific IgG responses 8–10 weeks after full vaccination, compared to convalescent subjects. Additionally, SARS-CoV-2-neutralizing IgA was considered as a critical component of the antiviral immune component [18,19]. Nevertheless, SARS-CoV-2-specific IgA responses are suboptimal among most vaccinee recipients, suggesting that the formulation and delivery approach of next-generation COVID-19 vaccine might be further optimized to induce the mucosal immunity. Besides, the vaccinee sera showed reduced levels of neutralizing ability against B.1.617.1 and other circulating variants, highlighting the urgent need for booster doses beyond the conventional two-dose regimen.

We observed a notable expansion of long-lasting, isotype-switched IgG^+ memory B cells among virus-specific memory B cells following vaccinations, lasting for at least 6–8 weeks. Indeed, SARS-CoV-2 infection-induced memory B cells are durable and long-lived for at least 8 months post disease onset [10,20]. Our data indicate that sustained memory B cells might be important for durability of anti-SARS-CoV-2 immunity and potential recall responses to infection or future boost.

Beyond humoral responses, successful protection against infectious diseases can be accomplished by alternative adaptive immune responses, including $CD4^+$ T cells, $CD8^+$ T cells and their corresponding memory subsets [21,22]. SARS-CoV-2-specific $CD4^+$ T cells and $CD8^+$ T cells were associated with reduced disease severity [4,23]. Potent memory $CD4^+$ and $CD8^+$ T cell responses were also detected from vaccinees, and the magnitudes were comparable to those in convalescent patients. Further, a prominent population of $CD4^+$ and $CD8^+$ memory T cells were biased toward T_{EMRA} and T_{EM} cells. These favourable phenotypes were considered as cytotoxic and long-lived with the potential to respond rapidly to eliminate the infected cells [13,24].

Age and interval might account for the heterogeneity of adaptive immune responses elicited by full vaccination with CoronaVac. As widely observed in COVID-19 patients, age correlated with COVID-19 disease severity, which might be associated with a low percentage of naïve $CD4^+$ and $CD8^+$ T cells [23]. Here we also observed a trend that the quality of vaccine-elicited immune response deteriorates with age, especially for neutralizing activities [25]. In addition, the dosing interval >21 days was beneficial for robust SARS-CoV-2-specific adaptive responses. Consistently, extended interval vaccination for BNT162b2 could boost the peak antibody responses in older individuals, which might be critical to further optimize the vaccine regimen for provision of effective and sustained immunity [26].

Very few published datasets compared antigen-specific antibody, B cells, $CD8^+$ T cells and $CD4^+$ T cells following vaccination in the same individuals. For those vaccinees who failed to generate neutralizing antibodies, robust dpike-specific memory B cells, SARS-CoV-2 memory $CD4^+$ and $CD8^+$ T cells were detected at a similar magnitude as those in NAb responders. Whether these specific $CD4^+$ and $CD8^+$ T cells could also serve as surrogates for protective immunity remains to be determined. Meanwhile, we also identified SARS-CoV-2 memory $CD4^+$ T cells strongly associated with RBD-specific memory B cells as well as SARS-CoV-2 memory $CD8^+$ T cells, indicating a convergent development of humoral and cellular adapative immunity.

This study has some limitations. The follow-up observation time in our study was relative short, only extending up to 8–10 weeks post full vaccination. Besides, the alternative function of vaccine-elicited antibody such as antibody-dependent cell-mediated cytotoxicity (ADCC) [27] were not evaluated.

In summary, this study demonstrated multiple compartments of adaptive immunity elicited by an authorized inactivated vaccine in an integrated manner. Our study offers insight into the underlying immunobiology of inactivated virus vaccines in humans and may have implications for vaccine strategies in the future.

Author contributions

CW, HS and YC designed the study. YC, YT, YY and YX recruited the patients. JP and JN processed the blood samples, ML, YS and YW performed cellular analysis. TX and MM performed the antibody assay. RH, XY and HS analysed and interpreted the data. YC, SY and TX wrote the manuscript. All the authors revised the manuscript.

Transparency declaration

The authors declare that they have no conflicts of interest. This study was supported by Clinical Trials from the Affiliated Drum Tower Hospital, Medical School of Nanjing University (2021-LCYJ-PY-10), Nanjing Medical Science and Technique Development Foundation (QRX17141, YKK19056, YKK20058 and YKK20076), National Natural Science Foundation of China (82002133), and

Please cite this article as: Chen Y et al., Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.10.006

Fundamental Research Funds for the Central Universities (14380459).

Acknowledgements

We thank all vaccine recipients for providing serum samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.10.006.

References

[1] Jara A, Undurraga EA, Gonzalez C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. N Engl J Med 2021;385:875–84.
[2] Braun J, Loyal L, Frentsch M, Wendisch D, Georg P, Kurth F, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature 2020;587:270–4.
[3] Brouwer PJM, Caniels TG, van der Straten K, Snitselaar JL, Aldon Y, Bangaru S, et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. Science 2020;369:643–50.
[4] Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell 2020;181:1489–1501 e15.
[5] Hartley GE, Edwards ESJ, Aui PM, Varese N, Stojanovic S, McMahon J, et al. Rapid generation of durable B cell memory to SARS-CoV-2 spike and nucleocapsid proteins in COVID-19 and convalescence. Sci Immunol 2020;5.
[6] Okba NMA, Muller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease patients. Emerg Infect Dis 2020;26:1478–88.
[7] Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. Cell 2021;184:861–80.
[8] Karim SSA. Vaccines and SARS-CoV-2 variants: the urgent need for a correlate of protection. Lancet 2021;397:1263–4.
[9] Krammer F. Correlates of protection from SARS-CoV-2 infection. Lancet 2021;397:1421–3.
[10] Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021:371.
[11] Chen Y, Shen H, Huang R, Tong X, Wu C. Serum neutralising activity against SARS-CoV-2 variants elicited by CoronaVac. Lancet Infect Dis 2021;21:1071–2.
[12] Chen Y, Tong X, Li Y, Gu B, Yan J, Liu Y, et al. A comprehensive, longitudinal analysis of humoral responses specific to four recombinant antigens of SARS-CoV-2 in severe and non-severe COVID-19 patients. PLoS Pathog 2020;16:e1008796.
[13] Tian Y, Babor M, Lane J, Schulten V, Patil VS, Seumois G, et al. Unique phenotypes and clonal expansions of human CD4 effector memory T cells re-expressing CD45RA. Nat Commun 2017;8:1473.
[14] Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 1999;401:708–12.
[15] Goel RR, Apostolidis SA, Painter MM, Mathew D, Pattekar A, Kuthuru O, et al. Distinct antibody and memory B cell responses in SARS-CoV-2 naive and recovered individuals following mRNA vaccination. Sci Immunol 2021;6.
[16] Favresse J, Bayart JL, Mullier F, Elsen M, Eucher C, Van Eeckhoudt S, et al. Antibody titres decline 3-month post-vaccination with BNT162b2. Emerg Microbes Infect 2021;10:1495–8.
[17] Kaneko N, Kuo HH, Boucau J, Farmer JR, Allard-Chamard H, Mahajan VS, et al. Loss of Bcl-6-expressing T follicular helper cells and germinal centers in COVID-19. Cell 2020;183:143–57. e13.
[18] Sterlin D, Mathian A, Miyara M, Mohr A, Anna F, Claer L, et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. Sci Transl Med 2021;13:eabd2223.
[19] Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Viant C, Gaebler C, et al. Enhanced SARS-CoV-2 neutralization by dimeric IgA. Sci Transl Med 2021;13:eabf1555.
[20] Rodda LB, Netland J, Shehata L, Pruner KB, Morawski PA, Thouvenel CD, et al. Functional SARS-CoV-2-specific immune memory persists after mild COVID-19. Cell 2021;184:169–83. e17.
[21] Zhao J, Zhao J, Mangalam AK, Channappanavar R, Fett C, Meyerholz DK, et al. Airway memory CD4(+) T cells mediate protective immunity against emerging respiratory coronaviruses. Immunity 2016;44:1379–91.
[22] Masopust D, Soerens AG. Tissue-resident T cells and other resident leukocytes. Annu Rev Immunol 2019;37:521–46.
[23] Rydyznski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D, et al. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. Cell 2020;183:996–1012. e19.
[24] Tian Y, Babor M, Lane J, Seumois G, Liang S, Goonawardhana NDS, et al. Dengue-specific CD8+ T cell subsets display specialized transcriptomic and TCR profiles. J Clin Invest 2019;129:1727–41.
[25] Ciabattini A, Nardini C, Santoro F, Garagnani P, Franceschi C, Medaglini D. Vaccination in the elderly: the challenge of immune changes with aging. Semin Immunol 2018;40:83–94.
[26] Parry H, Bruton R, Stephens C, Brown K, Amirthalingam G, Hallis B, et al. Extended interval BNT162b2 vaccination enhances peak antibody generation in older people. medRxiv 2021. 2021.05.15.21257017.
[27] Geers D, Shamier MC, Bogers S, den Hartog G, Gommers L, Nieuwkoop NN, et al. SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. Sci Immunol 2021;6.

Please cite this article as: Chen Y et al., Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.10.006

1.11. CoronaVac induz respostas de anticorpos rápidas e duradouras por até 12 meses, afirma estudo

Um estudo científico publicado por pesquisadores chineses do Centro de Controle e Prevenção de Doenças e da Universidade Médica Capital, ambos de Pequim, evidencia que a resposta imune humoral e celular induzida pela CoronaVac, vacina do Butantan e da Sinovac contra a Covid-19, permanece por um ano no organismo. O trabalho foi submetido à conceituada publicação médica britânica The Lancet, tendo sido publicado sob a forma de preprint.

Foram analisados 150 voluntários, com idades entre 18 e 59 anos, que receberam as duas doses da vacina com 14 dias de intervalo. Para poder verificar a evolução do panorama imunológico dos participantes, amostras de sangue foram coletadas antes do recebimento da primeira dose da vacina, assim como decorridos um, três, seis e 12 meses após a segunda dose.

Os cientistas constataram que, um mês após a imunização completa, os anticorpos de ligação e os anticorpos neutralizantes surgiram rapidamente. A taxa soropositiva de anticorpos de ligação foi de 99% e a taxa de anticorpos neutralizantes foi de 50%. Do terceiro até o 12º mês após a imunização, houve uma ligeira diminuição ao longo do tempo nos anticorpos neutralizantes e anticorpos de ligação. Aos 12 meses, porém, os anticorpos ainda eram detectáveis.

Em termos mais técnicos, a secreção de interferon-gama (IFN-γ) e da interleucina 2 (IL-2) induzida especificamente por RBD (domínio de ligação ao receptor) persistiram em níveis elevados por até seis meses, e puderam ser observadas ao longo dos 12 meses de análise. Além disso, células CD4 + TCM, CD4 + TEM, CD8 + TEM e CD8 + TE específicas para SARS-CoV-2 foram todas detectáveis e funcionais por até 12 meses após a administração da segunda dose.

Assim, os pesquisadores chineses constataram a persistência da resposta imune induzida pela Coronavac em um regime de duas doses. Foi comprovado que a vacina não apenas induziu ligações duráveis e respostas de anticorpos neutralizantes, como também células T de memória CD4 + e CD8 + específicas para SARS-CoV-2 por até 12 meses.

Publicado em: 19/10/2021

Status of Humoral and Cellular Immune Responses within 12 months Following CoronaVac Vaccination against COVID-19

Wei Zhao, Wei-xin Chen, Juan Li, Meng Cheng, Qin Li, Min Lv, Shan-Shan Zhou, Shuang Bai, Ya-li Wang, Li-chi Zhang, Peng Zhang, Jian Wang, Qun Zheng, Jiang Wu

Experimental Center for Basic Medical Teaching, School of Basic Medical Sciences, Capital Medical University (Q Zheng PhD); **Beijing Center for Disease Prevention and Control, Beijing Research Center for Preventive Medicine** (W Zhao PhD, W Chen MSc, J Li PhD, M Cheng MSc, Min Lv PhD, S Zhou MSc, S Bai MSc, Y Wang MSc, L Zhang MSc, P Zhang PhD, J Wang PhD, Prof J Wu); **Department of Laboratory, Yanjing Medical College, Capital Medical University** (Q Li PhD**).**

Correspondence to:

Prof Qun Zheng, Experimental Center for Basic Medical Teaching, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China

zhengqun@ccmu.edu.cn

or

Prof Jiang Wu, Beijing Center for Disease Prevention and Control, No.16, Hepingli middle street, Dongcheng District, Beijing, 100013, China.

wj81732@hotmail.com

Summary

Background Understanding immune memory to COVID-19 vaccines is critical for the design and optimal vaccination schedule for curbing the COVID-19 pandemic. Here, we assessed the persistence of humoral and cellular immune responses for 12 months after two-dose CoronaVac.

Methods Participants aged 18–59 years received two doses of 3 μg CoronaVac 14 days apart, and blood samples were collected before vaccination (baseline) and at 1, 3, 6, and 12 months after the second shot. Humoral responses of specific antibodies and neutralising antibodies were measured by using chemiluminescent immunoassay and wild-type SARS-CoV-2 microneutralisation assay, respectively. Cellular responses were measured by immunospot-based and intracellular cytokine staining assays. This trial is registered with ClinicalTrials.gov, NCT05072496.

Findings Total 150 participants were enrolled, and 136 of them completed the study through the 12-month endpoint. At 1 month after vaccination, binding and neutralising antibodies emerged rapidly, the seropositive rate of binding antibodies and seroconversion rate of neutralizing antibodies was 99% and 50%, respectively. From 3 to 12 months, the binding and neutralizing antibodies declined slightly overtime. At 12 months, the binding and neutralizing antibodies were still detectable and significantly higher than the baseline. IFN-γ and IL-2 secretion specifically induced by RBD persisted at high levels until 6 months, and could be observed at 12 months, while the levels of IL-5 and Granzyme B were hardly detected, demonstrating a Th1-biased response. Besides, specific $CD4^+$ T_{CM}, $CD4^+$ T_{EM}, $CD8^+$ T_{EM} and $CD8^+$ T_E cells were all detectable and functional up to 12 months after the second dose, as the cells produced IFN-γ, IL-2, and GzmB in response to stimulation of SARS-CoV-2 RBD.

Interpretation CoronaVac not only induced durable binding and neutralising antibody responses, but also SARS-CoV-2-specific $CD4^+$ and $CD8^+$ memory T cells for up to 12 months.

Funding Beijing Municipal Science & Technology Commission

Research in context

Evidence before this study

We searched PubMed for clinical trials published from the inception of the database to Oct 8, 2021, with the search terms “SARS-CoV-2”, “vaccine”, and “immune persistence”; no language restrictions were applied. We initially identified 206 references but this number decreased to 11 when we included the term “clinical trial”. Of these references, 3 of which report human clinical trials of SARS-CoV-2 vaccines. In the first study, six healthcare workers who contracted SARS-CoV-2 received the BNT162b2 mRNA COVID-19 vaccine, and had markedly higher neutralizing antibodies than those infected naturally. In the second study, 54 participants with HIV received two doses of ChAdOx1 nCoV-19, and there is no difference in magnitude or persistence of SARS-CoV-2 spike-specific humoral or cellular responses compared with participants without HIV. In the third study, the titrate of SARS-CoV-2 spike-specific IgG at day 320 after receiving a single dose of AstraZeneca ChAdOx1 declined to less than a third of the peak level, although the levels remained higher than the baseline. In the same study, a third injection boosted antibodies to a level that correlated with high efficacy after the second dose and boosted T-cell responses as well.

Added value of this study

To our knowledge, the present study is the first to report clinical data about immune persistence of an inactivated COVID-19 vaccine, which was monited for 12 months. Specific binding and neutralising antibodies peaked at 1 month after the second shot, and then dropped overtime, but remained significantly higher than baseline at 12 months. ELISpot responses showed that cytokine secretion was heavily biased toward to Th1 (IFN-γ and IL-2) rather than Th2 (IL-5) pathway, indicating that CoronaVac mainly induced a Th1-biased cellular immune response. Additionally, IFN-γ- or IL-2-producing $CD4^+$ and $CD8^+$ T cells were noted and detectable throughout the full observation period of 12 months following the boost.

Implications of all the available evidence

The CoronaVac, an inactivated COVID-19 vaccine, induced durable humoral and cellular immune responses for 12 months after the second shot, which would be valuable in restricting the COVID-19 pandemic. The mechanism of immune memory for the inactivated COVID-19 vaccine, of course, needs further investigation.

Introduction

COVID-19 is a worldwide emergency.[1] The urgent need for safe and effective interventions to mitigate the global spread of SARS-CoV-2 has prompted international efforts to develop vaccines. As of Oct 8, 2021, twenty-four COVID-19 vaccines have been approved for use[2] and more than 6.44 billion doses have been administered.[3] However, compared with other vaccines, the time interval between research and development and application of COVID-19 vaccines is very short, the underlying immunological mechanisms are not well-understood, such as antibody persistence, immune memory, etc. Therefore, it is important that more follow-up studies need to investigate the kinetics of neutralising antibody and immune memory of T and B cells, which will not guide the design of vaccination schedule, but also improve efficacy of vaccines.

CoronaVac (Sinovac Life Sciences, Beijing, China) is an inactivated vaccine against COVID-19, which has been currently approved for emergency use in China[4], and has also been included in the World Health Organization's (WHO) emergency use listing.[5] The data derived from phase 1-3 trials have shown that inactivated COVID-19 vaccines are effective, immunogenic and safe in children and adolescents aged 3–17 years,[6] and adults aged 18 years and older.[4] Here, we reported the status of persistence of antibodies and cellular responses within 12 months after two-dose of CoronaVac.

Methods

Study design, participants and collection of samples

The prospective cohort study was performed to evaluate the immunogenicity of an inactivated COVID-19 vaccine (CoronaVac, Sinovac Life Sciences, Beijing, China) in adults aged 18–59 years and followed up for 12 months after two vaccinations.
This trial was run at Beijing Center for Disease Prevention and Control (CDC), China. Participants who were healthy, non-pregnant adults 18-59 years of age were recruited from staff at Beijing CDC and Huairou District CDC (Beijng, China). All participants

provided written informed consent before enrolment. The trial protocol was approved by the Ethics Committee of Beijing CDC (2020-28) and was performed in accordance with the requirements of Good Clinical Practice of China and the International Conference on Harmonisation. The main exclusion criteria included history of SARS-CoV, SARS-CoV-2, or Middle East respiratory syndrome infection, high-risk epidemiology history within 14 days before enrolment (eg, travel or residence history in communities with case reports, or contact history with someone infected with SARS-CoV-2), axillary temperature of more than 37·0°C, history of allergy to any vaccine component. A complete list of exclusion criteria is in the protocol.
The participants were administered 3 µg CoronaVac intramuscularly following a 2-shot vaccine schedule, 14 days apart. Following that, the samples, including serum, plasma, and peripheral blood mononuclear cells were collected for investigation of exploratory end.

Procedures

CoronaVac, an inactivated vaccine containing whole-virion SARS-CoV-2, was developed by Sinovac Life Sciences (Beijing, China), and has been approved in 40 countries for emergency use as of Sep 15, 2021.[4,7] Using a 2-dose regimen, the participants received CoronaVac intramuscularly on day 0 and day 14, respectively. Blood samples were collected from participants on the day 0 before vaccination (baseline) and at 1, 3, 6, and 12 months after the second shot for analysing immunogenicity of vaccination.

The commercial chemiluminescence detection kits (2019-nCoV IgG antibody detection kit, Bioscience Diagnostics, Tianjin, China) were employed to measure SARS-CoV-2 receptor-binding domain (RBD) specific IgG following manufacturer's instructions as described before.[8] The titrates of neutralising antibodies against live SARS-CoV-2 (virus strain: SARS-CoV-2/human/CHN/CN1/2020, GenBank number MT407649.1) were quantified using the micro cytopathogenic effect assay[6]. All procedures related to virus neutralisation test were performed in a level 3 biosafety laboratory from Sinovac

Life Sciences, following WHO recommendations.

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood samples before vaccination and at month 1, 3, 6, and 12 post-vaccination. Enzyme-linked immunospot (ELISpot) assays (Cellular Technology Limited, OH, USA) were used to evaluate cellular immune responses through measuring expression of interferon (IFN) γ, interleukin-2 (IL-2), IL-5 by PBMS stimulated with RBD according to manufacturer's standard protocol. All measurements were subtracted by the unstimulated control values, while the subtracted values were corrected to zero. In addition, Flow cytometry (BD FACSLyric™, CA, USA) was employed to analyze proportions of the $CD4^+$ memory T-cell and $CD8^+$ memory T-cell subsets. Furthermore, intracellular production of IFN-γ, IL-2, and Granzyme B (GrzB) by T cells stimulated with RBD was also analyzed using flow cytometry as previously described. [9,10] The data were analysed with FlowJo software (Ashland, OR, USA).

Outcomes

Overall objectives were to assess the durability of the SARS-CoV-2-specific immune responses after CoronaVac vaccination as two intramuscular doses 14 days apart for up to 12 months. The humoral immunogenicity outcomes include the titres of RBD-specific IgG antibodies and neutralising antibodies against live SARS-CoV-2 at baseline and 1, 3, 6, and 12 months after the second shot of the vaccination. The positive cutoff value for RBD-specific IgG antibodies was defined as the sample cutoff (S/CO) value ≥1.0. Seroconversion of neutralising antibodies was defined as a titer of 8 or higher for neutralizing antibodies to live SARS-CoV-2. The cellular immune response outcomes include ELISpot assays for measuring secretion of IFN-γ, IL-2, IL-5, and GrzB by PBMS. The results are expressed as the number of spot-forming cells (SFCs) per 1,000,000 cells. In the meanwhile, the proportion of memory T-cell responses was also measured by ICS assays across as the above time points of the blood collection.

Statistical analysis

The sample size for this study was based on practical considerations rather than statistical power calculations. The data of immunogenecity were analysed descriptively using SAS (version 9·4). Titres of specific binding antibodies against SARS-CoV-2 RBD were presented as sample cutoff values (S/CO) with 95% CIs. Efficacy of neutralising antibodies was prensented as geometric mean titres (GMTs) with 95% CIs. Cellular immune responses were presented as the number of spot-forming cells (SFCs) per 1 million cells or as a proportion of positive responders with 95% CIs. The geometric means were calculated with $\log_{10}$ values of the original data, then the two-sided 95% CIs were calculated using Student's *t* distribution, with subsequent antilog transformation applied. χ^2 test was used to analyse categorical data, and ANOVA test was used to analyse numerical data. When the overall difference across the five time points was significant, paied t-test was used to compare the differences between groups. Two-sided p-values of less than 0.05 were considered significant. Figures were made using GraphPad Prism 8.0.1.

This study is registered with ClinicalTrials.gov, NCT05072496.

Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Total 150 participants were enrolled this study. Among them, 145 participants received two dose of the investigational product, and 136 participants completed the scheduled visits 12 months after the second shot. Baseline demographic characteristics of the participants at enrolment were shown in figure1.

Chemiluminescent immunoassay (CLIA) showed that at baseline, none of the participants had any detectable RBD-specific IgG antibody (figure 2). At 1month after the second vaccination, titers of RBD-specific IgG antibodies were strikingly enhanced

to a maximum S/COvalue of 11·26 (95% confidence interval [CI], 9·29to 13·24), and the seropositiverate was 99% (141 of 143 participants). Although the mean concentration of the RBD-specific IgG antibodies at 3 months (S/CO value 3·87, [95% CI 2·85–4·90]) was only one third of the peak level observed at the 1month, the seropositive rate still persisted at a high level (92%, 130 of 142). Thereafter, the antibody titers reached a plateau phase with only a gradual decline from 3 to 12 months (6 months S/CO value 3·68, [95% CI 2·43–4·94]; 12 months S/CO value 2·11, [95% CI 1·50–2·72]). The seropositive rates of RBD-specific IgG antibody were 77% (105 of 136) and 49%(67 of 136) at 6 and 12 months after the second vaccination, respectively.

As expected, there were no detectable titres of neutralising antibodies in serua of all study participants at baseline (figure 2). At 1month after the second vaccination, neutralising antibody titres increased substantially from baseline to a geometric mean titre (GMT) with peak level of 7·0 (95% CI 4·9–9·1), while the seroconversion rate was 50% (71of 143 participants). Similar to RBD-specific IgG antibody, at 3 months after the second vaccination, a rapid decline in GMT of neutralising antibody (4·4, 95% CI 2·3–6·4) was observed, followed by a plateau phase. Interestingly, GMT of neutralizing antibody did not decrease continuously at 6 months, but increased significantly compared with that at 3 months, reaching 5·3 (95% CI 3·1–7·4). At 12 months, GMT of the neutralising antibody decreased to 4·1 (95% CI 2·0–6·2), yet remained significantly higher than the baseline, and which there was no significant difference between the GMT of 3 months and 12 months after the second vaccination. The seroconversion rates of neutralising antibody at 3, 6, and 12 months were 21% (29 of 140), 35% (48 of 136), and 20% (27 of 136), respectively, which were consistent with the changing trend of neutralising antibody titres.

SARS-CoV-2 RBD-specific IFN-γ, IL-2, IL-5, and GrzB ELISpot responses were assessed at 1, 3, 6, and 12 months after the second vaccination in PBMCs of all participants (figure 3). IFN-γ responses were elicited in participants with a peak

frequnce (SFCs 1107·7, [95% CI 941·1-1274·3]) at 1 month after the second vaccination, and stabilized towards 3 months (SFCs 1093.1, [95% CI 931·8-1254·5]). Although some decline in SFCs was seen, relative high levels of IFN-γ responses persisted to 6 months (SFCs 772·6, [95% CI 614·6-930·7]). At 12 months, IFN-γ responses further declined but were still detectable (SFCs 123·3, [95% CI 64·5-182·2]). In addition, IL-2 responses were also noted at each time point after the second vaccination, and showed a similar pattern to IFN-γ responses: high levels of IL-2 responses persisted until the end of 6 months after the second vaccination. Although some participants had detectable IL-5 responses after vaccination, IL-5 responses were obviously lower than that of IFN-γ and IL-2 at each time point after vaccination, indicating a type 1 helper T-cell (Th1) biased cellular immune response. GrzB responses was not detecteble at each time point after vaccination.

Memory T-cell subsets, expression of IFN-γ, IL-2, and GrzB were ananlyzed by uisng ICS assays to evaluate the SARS-CoV-2 RBD-specific memory T cells in a subset of participants (N=119, in whom sufficient PBMC were available) (figure 4). The percentage of RBD-specific $CD4^+$ T central memory (T_{CM}) cells was significantly higher at 1 month (11·78%%) after the second vaccination than that of the baseline, repsenting 76% (86/113) of participants with detectable RBD-specific $CD4^+$ T_{CM} cells. Then, the fraction of RBD-specific $CD4^+$ T_{CM} cells slightly but significantly increased (15·25%) as compared with those of 1 month, declined until 6 months (1.97%), and stabilized towards 12 months (1·24%) after the second vaccination (figure 4). Coversingly, the percentages of subjects with detectable circulating SARS-CoV-2 RBD-specific $CD4^+$ T_{CM} cells were 86% (95 of 110), 59% (64 of 108), and 56% (65 of 117) at 3, 6, and 12 months after the second vaccination, respectively. In the meanwhile, the specific $CD8^+$ effector memory (T_{EM}) responses were also noted. A considerable fraction of RBD-specific CD8+ T_{EM} cells was observed at 1 month (9·48%), then the fraction of specific $CD8^+$ T_{EM} peaked at 3 months (12·14%),and thereafter dropped over time (6 months 5·73% and 12 months 0·89%). The proportion of subjects with detectable circulating SARS-CoV-2 RBD-specific $CD8^+$ effector memory (T_{EM}) cells

were 69% (78 of 113),78% (86 of 110), 56% (60 of 108), and 31% (36 of 117) of participants at 1, 3, 6, and 12 months after the last vaccination, respectively. Besides, we also observed that the fractions of $CD4^+$ T_{EM} and $CD8^+$ T_E cells specific to SARS-CoV-2 RBD increased over time and constituted up to about 7.51% of total peripheral blood $CD4^+$ T cells and about 8.74% of total peripheral blood $CD8^+$ T cells, respectively (figure 4).

As known, memory T cells, once they meet same antigen(s), can rapidly express a wide variety of cytokines to engage, recruit, or activate innate cells or other adaptive lymphocytes. To assess functionality of the SARS-CoV-2-specific memory $CD4^+$ and $CD8^+$ T cell responses, we further measured intracellular cytokines expressed by these cells in response to SARS-CoV-2 RBD stimulation (figure 4). IFN-γ cytokine-producing memory $CD4^+$ T and $CD8^+$ T cells exhibited similar kinetics, in which IFN-γ production started at 1 month, reached the peak at 3 or 6 months, and thereafter dropped over time (figure 4). It has been well known that GzmB is a type of cytotoxic granules produced by NK cells and activated CTLs.[11] As expected, the GzmB production by specific memory $CD4^+$ T and $CD8^+$ T cells increased rapidly at 1 month after the second vaccination, and maintained a high percentage to 3 months, and then gradually decreased. Interestingly, the fraction of $CD4^+$ T_{CM}, $CD4^+$ T_{EM}, $CD8^+$ T_{EM} , and $CD8^+$ $T_E$cells producing IL-2 continued to rise from 1 to 6 months after the second dose and maintained at a high level throughout the entire follow-up period (until 12 months). As shown in Fig4, the SARS-CoV-2-specific $CD4^+$ T_{cM}, $CD4^+$ T_{EM}, and $CD8^+$ T_{EM}, and $CD8^+$ T_E cells were all functional up to 12 months after the second dose, as the cells produced IFN-γ, IL-2, and GzmB in response to SARS-CoV-2-specific RBD. Therefore, CoronaVac is not only albe to elicit durable SARS-CoV-2-specific memory $CD4^+$T cells, but also SARS-CoV-2-specific memory $CD8^+$ T cells.

Dicussion

In the present study, we monitored the status of 12-month durability of humoral and cellular immune responses in 145 individuals who received two doses of CoronaVac (3

μg/per dose, with an interval of 14 days). Our findings extended previously reported results[4] and showed that SARS-CoV-2 RBD-specific binding and neutralisation antibody responses to immunozazition with CoronaVac decreased gradually with timebing, but remained significantly higher than baseline after 12 months. More importantly, it is the first time that status of robustly expanded SARS-CoV-2 RBD-specific memory $CD4^+$ and $CD8^+$ T cells in the peripheral circulation were monited through 12 months post booster vaccination. Furthermore, ELISpot responses and ICS used to characterize T cell cytokine responses showed that profile of cytokine secretion was mainly toward to Th1 (IFN-γ and IL-2) rather than Th2 (IL-5) pathway, suggesting that CoronaVac predominantly induces Th1-biased cellular immune responses. In addition, it is also worth to note that CoronaVac induced rapid and durable antibody responses as well as cellular immune responses for up to 12 months.

It is no doubt that understanding the duration of antibody responses to COVID-19 vaccine is the key to continuously prevent infection. Although correlates of protection against SARS-CoV-2 infection in human are not yet established,[12] the data od CLIA and micro cytopathogenic effect assay showed that binding and neutralizing antibodies elicited by two doses of CoronaVac were able to persist through 12 months after the second shot, indicating that CoronaVac has the potential to provide durable humoral immunity. However, to our knowledge at the moment, there are the limited data available showing that humoral responses to COVID-19 vaccines can last for the 12 months. It has been shown that the Moderna mRNA-1273 vaccine (the 100-μg per dose) produces high levels of binding and neutralizing antibodies that declined slightly overtime until 90 days after the booster vaccination.[12,13] Besides, a significant trend of waning antibody levels with time has been oberved in both AstraZeneca ChAdOx1 and Pfizer BNT162b2, with antibody levels reducing by about five-fold for ChAdOx1, and by about two-fold for BNT162b2, between 21–41 days and 70 days or more after the second dose, respectively.[14] At 320 days, titres of SARS-CoV-2 spike protein-specific IgG in AstraZeneca ChAdOx1 declined to less than a third of the peak titres, although it remained higher than the baseline after receivinga single dose of 5×10^{10} viral particles

booster vaccine.[15] Numerically, the humoral responses of CoronaVac are not as strong as other COVID-19 vaccines, however, we shoule bear that in our mind, i.e., it is difficult to directly evaluate the capcacies for producing antibodies among different vaccines without a head-to-head comparison due to heterogeneity of neutralization assays. Even though the same live virus is used for neutralization analysis, the results vary from laboratory to laboratory due to the lack of standardized laboratory methods for SARS-CoV-2 neutralization and experimental procedures, including virus titration, serum dilution, virus-serum neutralization, readout, and reporting methods.[16] Additionally, the relatively low humoral responses of CoronaVac in the present study might be associated with the relatively short vaccination schedule used. It has been shown that a more robust antibody response can be generated by the day 0 and 28 vaccination schedule as compared to the day 0 and 14 schedule. We current use, therefore, the day 0 and 28 vaccination as routine for CoronaVac.[4,8]

Although recent work has much focused on antibody responses, memory $CD8^+$ T cells play cruitical role in defencing virus infection through killing virus-infected cells and expressing relevant cytokines and cytolytic molecules.[17] In addition, $CD8^+$ T-cell responses may also contribute to protection, particularly in the setting of waning or borderline antibody responses,[18] or potentially against viral variants that are partially resistant to antibodies.[19] Previous studies on SARS and Middle East respiratory syndrome (MERS) have shown that the increases in specific antibodies are temporaryly, and that antibody levels decline quickly in patients after recovery, whereas the specific $CD4^+$ and $CD8^+$ T-cell responses play an essential role in the control of SARS and MERS.[20,21] Besides, some studies have shown that the reduction in the number of T cells is related to poor clinical outcomes and immune pathogenesis, while adequate T cell counts and appropriate effector function are associated with patients having mild disease symptoms or successful rehabilitation.[22] Grifoni et al. have reporte that circulating SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells are 100% and 70% respectively in a small group of COVID-19 convalescent patients (n=20).[23] a In addition, another study has shown that the percentages of $CD4^+$ and $CD8^+$ T cells

concomitantly increase from day 7 after infection, which persist for 7 days as the symptoms disappeared.[24] In contrast, in the present study we also interrogated the presence of functional $CD4^+$ and $CD8^+$ memory T cells in participants who received the vaccine. ELISpot results showed that RBD-specific T cells secreting IFN-γ and IL-2 persisted through 12 months after the second shot of vaccination. In the meanwhile, these SARS-CoV-2 RBD-specific memory $CD4^+$ and $CD8^+$ T cells still expressed detectable cytokines IFN-γ, IL-2, and GzmB throughout entire study duration. Together, these data demonstrate that CoronaVac are able to elicit SARS-CoV-2 RBD-specific memory $CD4^+$ and $CD8^+$ T cells, while these cells could be maintained and still have capacity producing effector cytokines after restimulation 12 months post boost. Although the classical immunological theory believes that the inactivated vaccines are not thought to induce CD8 T-cell responses, our data suggest that the structural integrity of whole SARS-CoV-2 might be the key to elicit antiviral $CD8^+$ memory T-cell responses. The exact mechanism behind this hypothesis, of course, needs further investigation.

Previous reports on the development of SARS and the Middle East respiratory syndrome (MERS) vaccine candidates have shown that there are some raised concerns related to antibody-dependant enhancement (ADE) and induction of Th2 responses.[25-27] In contrast, our data showed that profile of cytokine secretion was prodeminately Th1 (IFN-γ and IL-2) produced by BPBC stimulated with SARS-CoV-2 RBD compared to baseline of participants received CoronaVac, while concentrations of Th2 cytokine IL-5 were hardly detectable. Similarly, phenotyping by flow cytometry demonstrated that substantial IFN-γ- and IL-2-producing cells mainly were $CD4^+$ and $CD8^+$ T cells. Herein, subjects vaccinated with CoronaVac seemed to have predominant Th1 responses, but little to no Th2 cytokines. These results are consistent with a previous animal study,[28] and further proves the safety of CoronaVac.

However, it is notable that there are some limitations. First, because the participants involved in the study aged 18 to 59 years, the generalizability to those at risk for SARS-

CoV-2 infection and other regions requires to be further studied. Second, we did not perform a more in-depth T cell analysis before and after vaccination due to the limited volumes of blood samples available. Finally, due to the ethical issues, we could not assess the induction of tissue-resident memory T cells. These are being addressed by the ongoing clinical programme.

In conclusion, two-dose of CoronaVac not only induces durable binding and neutralization antibody responses, but also elicit SARS-CoV-2 RBD-specific memory $CD4^+$ and $CD8^+$ memory T cells for up to 12 months.

Contributors

All authors had full access to all data in the studies and had final responsibility for the decision to submit for publication. WZ, QL, PZ, QZ, and JW designed the study. QZ and JW worked as coprincipal investigators of this study. ML and LZ did the statistical analysis. WZ drafted the manuscript. QZ and JW critically reviewed and revised the manuscript. JL and SB led and participated in the site work, including the recruitment, follow-up, and data collection. WC, MC, SZ, SB, YW, and JW were responsible for laboratory analyses.

Declaration of interests

The authors declare that no competing interests exist.

Data sharing

We support sharing of the individual participant data. The individual participant data that underlie the results reported in this Article will be made available when the study is complete. Researchers who provide a scientifically sound proposal will be allowed access to the individual participant data. Proposals should be directed to the corresponding authors. These proposals will be reviewed and approved by the funder, investigator, and collaborators on the basis of scientific merit. To gain access, data requesters will need to sign a data access agreement.

Acknowledgments

This study was supported by the Beijing Municipal Science & Technology Commission (Z211100002521014). The authors would like to thank all the participants who volunteered for this study. We also wish to thank Prof Ying Sun (Capital Medical University School) for critical reading of this manuscript.

Reference

1. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**(8): 727-33.

2. uniecf. COVID-19 Vaccine Market Dashboard Accessed 8 Oct 2021. 2021.

3. Data Owi. https://ourworldindata.org/covid-vaccinations Accessed Oct 8, 2021. 2021.

4. Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21**(2): 181-92.

5. WHO. Background document on the inactivated vaccine Sinovac-CoronaVac against COVID-19: background document to the WHO interim recommendations for use of the inactivated COVID-19 vaccine, CoronaVac, developed by Sinovac, 24 May 2021. 2021.

6. Han B, Song Y, Li C, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021.

7. Sinovac: CoronaVac. https://covid19.trackvaccines.org/vaccines/7/.

8. Li J, Chen W, Chen M, Bai S, Yuan Q, Wu J. Immunogenicity of inactivated COVID-19 vaccines at different vaccination intervals. *Hum Vaccin Immunother* 2021; **17**(10): 3310-3.

9. Zhu FC, Li YH, Guan XH, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. *Lancet* 2020; **395**(10240): 1845-54.

10. Pan Y, Jiang X, Yang L, et al. SARS-CoV-2-specific immune response in COVID-19 convalescent individuals. *Signal Transduct Target Ther* 2021; **6**(1): 256.

11. Hiroyasu S, Zeglinski MR, Zhao H, et al. Granzyme B inhibition reduces disease severity in autoimmune blistering diseases. *Nat Commun* 2021; **12**(1): 302.

12. Widge AT, Rouphael NG, Jackson LA, et al. Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination. *N Engl J Med* 2021; **384**(1): 80-2.

13. Doria-Rose N, Suthar MS, Makowski M, et al. Antibody Persistence through 6 Months after the Second Dose of mRNA-1273 Vaccine for Covid-19. *N Engl J Med* 2021; **384**(23): 2259-61.

14. Shrotri M, Navaratnam AMD, Nguyen V, et al. Spike-antibody waning after second dose of BNT162b2 or ChAdOx1. *Lancet* 2021; **398**(10298): 385-7.

15. Flaxman A, Marchevsky NG, Jenkin D, et al. Reactogenicity and immunogenicity after a late second dose or a third dose of ChAdOx1 nCoV-19 in the UK: a substudy of two randomised controlled trials (COV001 and COV002). *Lancet* 2021; **398**(10304): 981-90.

16. Chen X, Chen Z, Azman AS, et al. Comprehensive mapping of neutralizing antibodies against SARS-CoV-2 variants induced by natural infection or vaccination. *medRxiv* 2021.

17. Rodda LB, Netland J, Shehata L, et al. Functional SARS-CoV-2-Specific Immune Memory Persists after Mild COVID-19. *Cell* 2021; **184**(1): 169-83 e17.

18. McMahan K, Yu J, Mercado NB, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature* 2021; **590**(7847): 630-4.

19. Stephenson KE, Le Gars M, Sadoff J, et al. Immunogenicity of the Ad26.COV2.S Vaccine for COVID-19. *JAMA* 2021; **325**(15): 1535-44.

20. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature* 2020; **586**(7830): 594-9.

21. Channappanavar R, Fett C, Zhao J, Meyerholz DK, Perlman S. Virus-specific memory CD8 T cells provide substantial protection from lethal severe acute respiratory syndrome coronavirus infection. *J Virol* 2014; **88**(19): 11034-44.

22. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest* 2020; **130**(5): 2620-9.

23. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* 2020; **181**(7): 1489-501 e15.

24. Braun J, Loyal L, Frentsch M, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature* 2020; **587**(7833): 270-4.

25. Lurie N, Saville M, Hatchett R, Halton J. Developing Covid-19 Vaccines at Pandemic Speed. *N Engl J Med* 2020; **382**(21): 1969-73.

26. Yong CY, Ong HK, Yeap SK, Ho KL, Tan WS. Recent Advances in the Vaccine Development Against Middle East Respiratory Syndrome-Coronavirus. *Front Microbiol* 2019; **10**: 1781.

27. Peeples L. News Feature: Avoiding pitfalls in the pursuit of a COVID-19 vaccine. *Proc Natl Acad Sci U S A* 2020; **117**(15): 8218-21.

28. Gao Q, Bao L, Mao H, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. *Science* 2020; **369**(6499): 77-81.

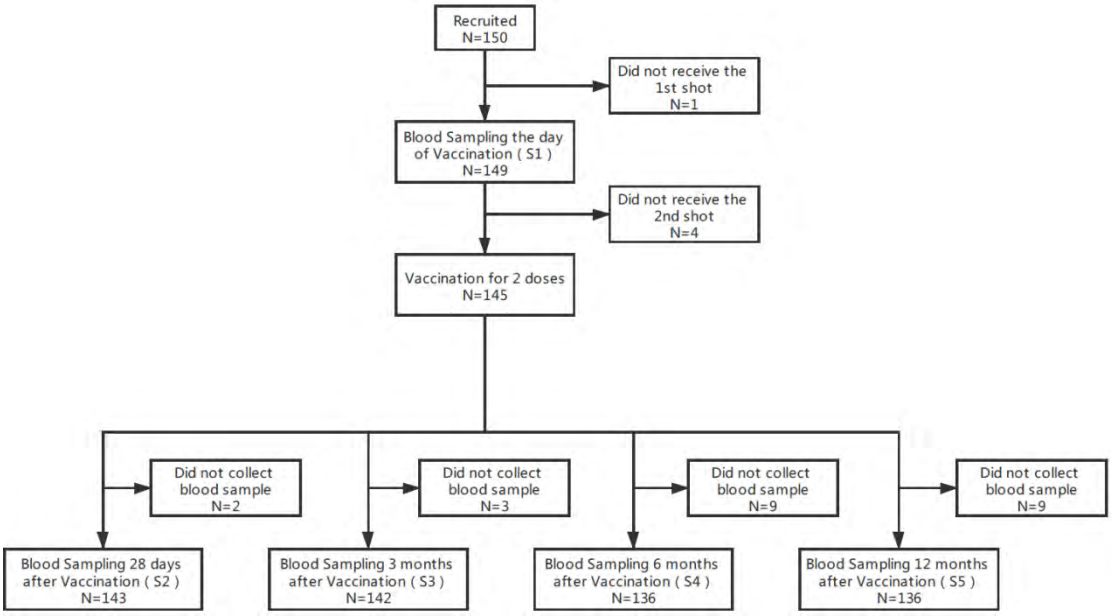


Figure 1: Design and Schedule of samples collection.

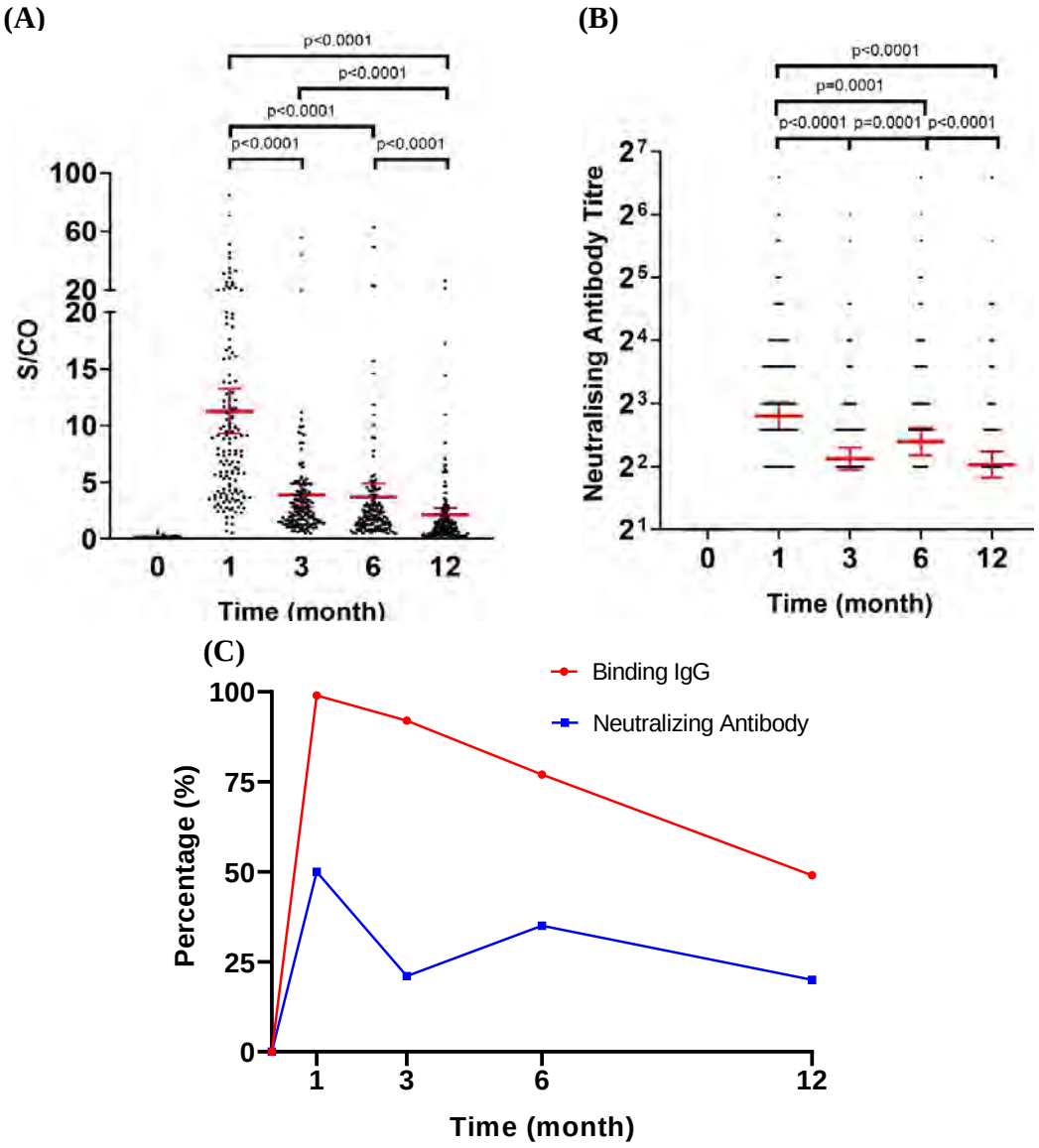


Figure 2: Status of sera IgG and neutralising antibody response following CoronaVac vaccination.

Spike RBD-binding IgG (A) and SARS-CoV-2 neutralising antibody (B) measured by CLIA and micro cytopathogenic effect assay. Participants received CoronaVac at day 0 and 14. Each data point represents a serum sample. The error bars of binding antibody are mean with 95% CI. The error bars of neutralising antibody are geometric mean with 95% CI. Seropositive rates of binding IgG and seroconversion rate of neutralising antibodies (C) were defined as S/CO value ≥1.0 and a titer of 8 or higher for neutralizing antibodies to live SARS-CoV-2, respectively. RBD=receptor binding domain

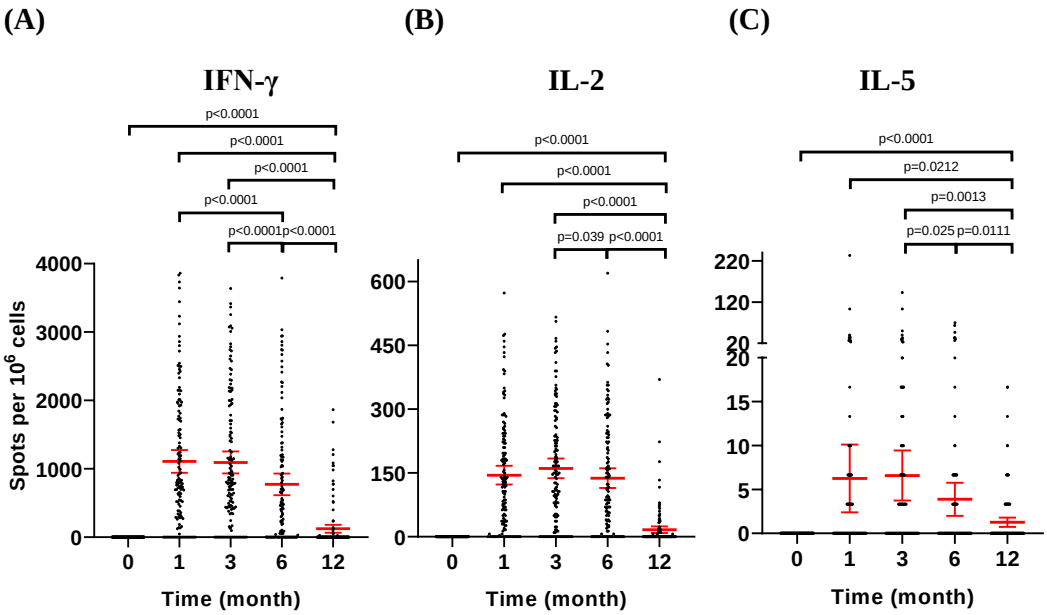


Figure 3: Status of specific T-cell responses following CoronaVac vaccination.

The number of specific T cells with secretion of IFN-γ, IL-2 and IL-5 of per million cells measured by ELISpot. Each data point represents the mean number of spots from triplicate wells for one participant, after subtraction of the unstimulated control. The error bars are geometric mean with 95% CI. IFN=interferon; IL=interleukin.

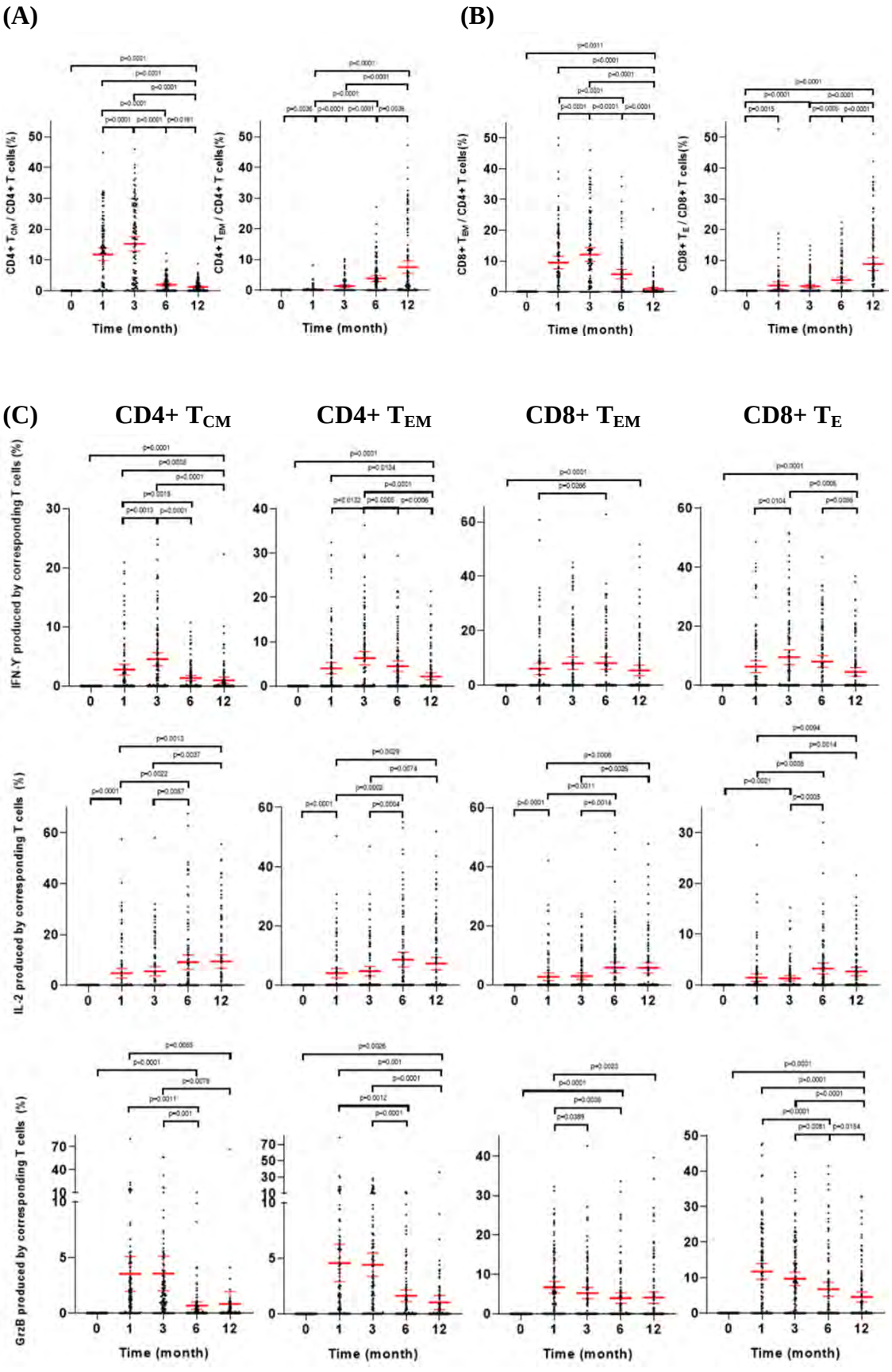


Figure 4 : Status of distribution and expression of cytokines by T_{CM} and T_{EM}

following CoronaVac vaccination.

(A) Percentage of T_{CM} and T_{EM} of total SARS-CoV-2-specific $CD4^+$ T cells. (B) Distribution of T_{EM} and T_E of total SARS-CoV-2-specific $CD8^+$ T cells. (C) Percentages of $CD4^+$ T_{CM}, $CD4^+$ T_{EM}, $CD8^+$ T_{EM}, and $CD8^+$ T_E cells expressed IFN-γ, IL-2, and GrzB responded specifically to RBD-stimulation. IFN=interferon; IL=interleukin; T_{CM}= central memory T cells; T_{EM}=effector memory T cells; T_E=terminal effector T cells. The error bars are geometric mean with 95% CI.

1.12. CoronaVac induz produção elevada de anticorpos neutralizantes, mostra estudo brasileiro

Um estudo publicado na revista Vaccines por pesquisadores da Universidade Estadual do Pará (UEPA) e da Universidade Federal do Pará (UFPA) demonstrou que a CoronaVac induz produção de anticorpos capazes de neutralizar o SARS-CoV-2 em mais de 70% dos imunizados, chegando a 93% em indivíduos entre 21 e 40 anos.

Os cientistas analisaram o soro de 358 residentes de Belém, no Pará, com idades entre 21 e 96 anos, sendo 138 homens e 220 mulheres. Todos foram vacinados com as duas doses da CoronaVac com um intervalo de 20 dias e as amostras de sangue foram coletadas entre março e abril de 2021.

Dos participantes, 205 fizeram testes para avaliar o total de anticorpos contra o SARS-CoV-2. Destes, 77,6% apresentaram soropositividade. Os outros 153 indivíduos testaram a presença de anticorpos neutralizantes específicos para o domínio de ligação ao receptor (RBD) e 72,6% tiveram resultado positivo.

Os títulos de anticorpos neutralizantes foram significativamente maiores em indivíduos mais jovens – 93% entre 21 e 40 anos, 76% entre 41 e 60 e 72% entre 61 e 80 anos –, o que pode estar associado à senescência do sistema imune, segundo os pesquisadores. No entanto, além da presença de anticorpos após a vacinação, a imunidade também está associada à resposta das células T e B de memória, que não pode ser detectada pelos testes sorológicos. Outras pesquisas já mostraram que a vacina do Butantan induz resposta significativa dessas células, responsáveis por detectar a presença do vírus e rapidamente reagir com a ativação das células de defesa e a produção de anticorpos.

Publicado em: 12/10/2021

vaccines

MDPI

Communication

Assessment of Anti-SARS-CoV-2 Antibodies Post-Coronavac Vaccination in the Amazon Region of Brazil

Carlos David Araújo Bichara [1,2,3], Maria Alice Freitas Queiroz [1], Ednelza da Silva Graça Amoras [1], Gergiane Lopes Vaz [2], Izaura Maria Vieira Cayres Vallinoto [1], Cléa Nazaré Carneiro Bichara [4], Isabella Pinheiro Costa do Amaral [2], Ricardo Ishak [1] and Antonio Carlos Rosário Vallinoto [1,3,*]

1 Laboratory of Virology, Institute of Biological Sciences, Federal University of Pará (UFPA), Belém 66075-110, PA, Brazil; bichara@amaralcosta.com.br (C.D.A.B.); alicefarma@hotmail.com (M.A.F.Q.); ednelza@hotmail.com (E.d.S.G.A.); ivallinoto@ufpa.br (I.M.V.C.V.); rishak@ufpa.br (R.I.)
2 Amaral Costa Diagnostic Medicine, Belém 66055-050, PA, Brazil; gergiane@amaralcosta.com.br (G.L.V.); isabella@amaralcosta.com.br (I.P.C.d.A.)
3 Graduate Program in Biology of Infectious and Parasitic Agents, Institute of Biological Sciences, Federal University of Pará (UFPA), Belém 66075-110, PA, Brazil
4 Center for Biological and Health Sciences, School of Medicine, Pará State University (UEPA), Belém 66087-870, PA, Brazil; cleacarneirobichara@gmail.com
* Correspondence: vallinoto@ufpa.br; Tel.: +55-91-3201-7587

Abstract: The present study evaluated the frequency of seropositivity for anti-SARS-CoV-2 (S1 and S2) total antibodies and anti-SARS-CoV-2 (receptor binding domain-RBD-S1) neutralizing antibodies in individuals vaccinated with the immunizing agent Coronavac. This was a cross-sectional study involving 358 individuals divided into two groups. Group 1 consisted of 205 volunteers who were tested for anti-SARS-CoV-2 total antibodies; group 2 consisted of 153 individuals tested for the presence of anti-SARS-CoV-2 neutralizing antibodies. Seropositivity was greater than 70% in both groups, although 17.6% and 20.9% of individuals showed no neutralizing or total antibody reactivity, respectively. The frequency of anti-SARS-CoV-2 total antibodies displayed a significantly different distribution between the sexes but not according to age. The frequency of anti-SARS-CoV-2 neutralizing antibodies was 93.3% (95% CI 68.1–99.8) in the age group from 21 to 40 years but significantly decreased with advancing age, and was 76.2% (95% CI 52.8–91.8) for 41 to 60 years, 72.5% (95% CI 62.8–80.9) for 61 to 80 years, and 46.7% (95% CI 21.3–73.4) for >80 years. Our results reveal a high prevalence of anti-SARS-CoV-2 total antibodies and anti-SARS-CoV-2 neutralizing antibodies in individuals who received both doses of the Coronavac vaccine, suggesting a lower effectiveness of the humoral immune response among those older than 60 years of age, which might be associated with senescence of the immune system.

Keywords: COVID-19; SARS-CoV-2; vaccine; Coronavac; antibody

Citation: Bichara, C.D.A.; Queiroz, M.A.F.; da Silva Graça Amoras, E.; Vaz, G.L.; Vallinoto, I.M.V.C.; Bichara, C.N.C.; Amaral, I.P.C.d.; Ishak, R.; Vallinoto, A.C.R. Assessment of Anti-SARS-CoV-2 Antibodies Post-Coronavac Vaccination in the Amazon Region of Brazil. *Vaccines* **2021**, *9*, 1169. https://doi.org/10.3390/vaccines9101169

Academic Editors: Ralph A. Tripp, Steven B. Bradfute and Scott Anthony

Received: 22 August 2021
Accepted: 30 September 2021
Published: 12 October 2021

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic that emerged in Wuhan, China in November 2019 [1] was accompanied by a series of concerns, questions, and lessons [2]. The conditions brought about by this virus triggered the most intensive race in the history of science worldwide to develop a vaccine capable of eliciting neutralizing antibodies against SARS-CoV-2 and reinfections by different variants of the virus [3], conferring protection against severe cases of coronavirus disease 2019 (COVID-19) [4]. New anti-SARS-CoV-2 vaccine development platforms using the following—inactivated virus, nonreplicating viral vector, subunit, viral-like particle, DNA, and mRNA—were implemented [5,6], generating hundreds of records of preclinical and phase II, III, and IV clinical studies [7]. Along with proposals for new technologies for the creation of anti-SARS-CoV-2 vaccines, there were also doubts and concerns [8], especially

regarding the safety and efficacy of the new platforms, which, until then, had not been applied to humans [9].

Parallel to advances in our understanding of the immunological mechanisms present in SARS-CoV-2 infection and in the modulation of COVID-19, phase II and III studies showed satisfactory and promising efficacy and safety results for different anti-SARS-CoV-2 vaccine platforms [5]. This has resulted in 14 vaccines that are in use in different countries thus far.

Zhang et al. [10] investigated the immunogenic characteristics of different vaccine platforms and reported that humoral and cellular immune responses differed when administered individually, with inactivated vaccines showing lower levels of neutralizing antibody and T cell responses. A preprint of a retrospective cohort study assessed the effectiveness of Vaxzevria and Coronavac vaccines for COVID-19 in Brazil and reported overall effectiveness against severe COVID-19 for Vaxzevria up to 89 years of age and for Coronavac up to 79 years of age [11].

In Brazil, the National Health Surveillance Agency (ANVISA), a government agency responsible for regulating pharmacological and immunobiological inputs, approved the definitive registration of the Pfizer and AstraZeneca vaccines in January 2021. Coronavac (Sinovac) and Janssen-CILAG vaccines have been approved only for emergency use [12].

The main question and reason for the different opinions and discussions is associated with the effectiveness of mass vaccination campaigns, especially with regard to the effectiveness of immunization in generating protective immunity and immunological memory. This dilemma highlights the importance of evaluating variables such as sex and age, as the immune response can exhibit different dynamics based on sex [13] and physiological senescence of the immune system with age [14]. In this context, serological studies have been carried out to assess the effectiveness of generating post-vaccination neutralizing antibodies at the population level [15].

The present study examined the frequency of anti-SARS-CoV-2 total antibodies specific to the S1 and S2 portions of the viral spike protein, as well as the presence of anti-SARS-CoV-2 (receptor binding domain (RBD-S1)) neutralizing antibodies, in two independent groups of individuals who sought care at the Amaral Costa Medicina Diagnóstica laboratory after receiving the second dose of the Coronavac vaccine.

2. Materials and Methods

2.1. Studied Samples

This was a cross-sectional study in which blood samples were collected from March to April 2021 and included 358 individuals (Table 1) of both sexes (138 males and 220 females) aged between 21 and 96 years (average 66.6 years). The persons involved in the study voluntarily sought care at the Amaral Costa Medicina Diagnóstica in the city of Belem, the capital of the State of Para (Northern Brazil), after their second dose of Coronavac (Sinovac Research and Development Co. Ltd., Haidian District, Beijing, China/Butantan, São Paulo, Brazil) for the purposes of confirming serological conversion. Those persons who presented evidence of previous vaccination (second dose) within 30 days were invited to participate in the study. The vaccination regimen adopted was two doses with a time interval of 20 days. Of the total number of individuals analyzed, we performed an anti-SARS-CoV-2 total antibody test (S1 and S2) for 205; 153 individuals were tested for the presence of anti-SARS-CoV-2 neutralizing antibodies (RBD-S1).

Table 1. Frequency of anti-SARS-CoV-2 total antibodies (S1 and S2) and anti-SARS-CoV-2 neutralizing antibodies (RBD-S1) after two doses of Coronavac in Belem, Para.

Sample	Vaccine	Test	N	Male	Female	Age (mean/SD)	Reagent (%)	Indeterminate (%)	Negative (%)
Group 1	Coronavac	Total antibodies	205	77	128	65.5/14.8	159 (77.6%)	03 (1.5%)	43 (20.9%)
Group 2	Coronavac	Neutralizing antibodies	153	61	93	65.4/14.6	111 (72.6%)	15 (9.8%)	27 (17.6%)
Groups 1 and 2	Coronavac	Total and neutralizing antibodies	358	138	220	65.4/14.7	270 (75.4%)	18 (5.0%)	70 (19.6%)

This project was submitted to and approved by the Human Research Ethics Committee of the Institute of Health Sciences of the Federal University of Pará (CAAE: 31800720.1.0000.0018) in compliance with the guidelines and regulatory standards for research involving human beings. Individuals who agreed to participate in the study signed an informed consent form.

2.2. *Ethical Aspects*

The study was approved by the Ethics and Research in Human Beings Committee of the Health Sciences Institute of the Federal University of Pará (CAAE: 31800720.1.0000.0018) in compliance with the guidelines and regulatory standards for research involving human beings, in accordance with the Declaration of Helsinki.

2.3. *Antibody Analysis*

Whole blood samples (5 mL) were collected in vacuum tubes without anticoagulant. Serum was separated by centrifugation. Investigation of anti-SARS-CoV-2 total antibodies (S1 and S2) was performed using a qualitative microparticle chemiluminescent immunoassay (CMIA) with the LIAISON® XL Analyzer automated platform (DiaSorin, Saluggia, Italy) following the manufacturer's recommendations. The reference ranges were non-reagent (<12 AU/mL), indeterminate (12.0 $\geq$ x < 15.0 UA/mL), and reagent (>15.0 AU/mL).

Anti-SARS-CoV-2 (RBD-S1) neutralizing antibodies were detected using the competitive enzyme immunoassay GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection kit (GenScript, Piscataway, New Jersey, USA) following the manufacturer's protocol. The approach, also known as the SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) kit, is a faster, easier, more scalable, and automatable alternative to traditional neutralizing antibody tests, such as the virus neutralization test (VNT), pseudovirus neutralization test (pVNT), and plaque reduction neutralization test (PRNT). The reference ranges used were non-reagent (<20%), indeterminate (20 $\geq$ x $\leq$ 29%), and reagent (>30%).

2.4. *Statistical Analysis*

Information on sex, age, and antibody status was tabulated in Excel software. The calculation of antibody frequencies was performed by direct counting, and the significance of comparisons between groups was assessed by chi-square and G tests [16] using the Bioestat version 5.3 program. Differences were considered statistically significant when the *p*-value was <0.05.

3. Results

When evaluating anti-SARS-CoV-2 total and neutralizing antibodies, 270 samples (75.4%) presented positive results, 70 (19.6%) were non-reagent, and 18 (5.0%) were indeterminate (Table 1).

Testing for anti-SARS-CoV-2 total antibodies (205 individuals) showed a positive result for 159 samples (77.6%), while 43 (20.9%) were non-reagent and 3 (1.5%) were indeterminate

(Table 1). Regarding anti-SARS-CoV-2 neutralizing antibodies (153 individuals tested), 111 samples (72.6%) presented a positive result, 27 (17.6%) were non-reagent, and 15 (9.8%) were indeterminate (Table 1).

The numbers of individuals according to sex in each age group were as follows: 21–40 years (F = 26 (74.3%) and M = 9 (25.7%)), 41–60 years (F = 36 (60%) and M = 24 (40%)), 61–80 years (F = 129 (58.3%) and M = 92 (41.7%)), and > 80 years (F = 29 (69%) and M = 13 (31%)).

The seropositivity profiles (reagent vs. non-reagent) according to the results of both tests revealed a significantly higher value ($p = 0.0022$) in females (80%) than in males (68%; Figure 1A). A similar result was observed (87% vs. 68%; $p = 0.0041$) for the 205 individuals who underwent testing for anti-SARS-CoV-2 total antibodies (Figure 1B). However, no significant differences between sexes were found for the frequency of anti-SARS-CoV-2 neutralizing antibodies (75% vs. 69%; Figure 1C).

The seropositivity profile according to the results of anti-SARS-CoV-2 total antibodies plus anti-SARS-CoV-2 neutralizing antibodies (Figure 1A) indicated significant differences from the pooled analysis of age groups ($p = 0.0084$). The highest frequency occurred in the age group of 21–40 years (91.4%; 95% CI 76.9–98.2), gradually decreasing as age increased to 83.3% (95% CI 71.5–91.7) for 41–60 years, 73.9% (95% CI 65.1–81.6) for 61–80 years, and 61.9% (95% CI 45.6–76.4) for >80 years. These significant differences were not observed when measuring anti-SARS-CoV-2 total antibodies (Figure 1B) but followed the same pattern when measuring only anti-SARS-CoV-2 neutralizing antibodies ($p = 0.0218$; Figure 1C); individuals aged 21 to 40 years showed 93.3% (95%CI 68.1–76.2) seropositivity, which decreased gradually with age to 76.2% (95%CI 52.8–91.8) for 41 to 60 years, 72.5% (95%CI 62.8–80.9) for 61 to 80 years, and 46.6% (95%CI 21.3–73.4) for >80 years.

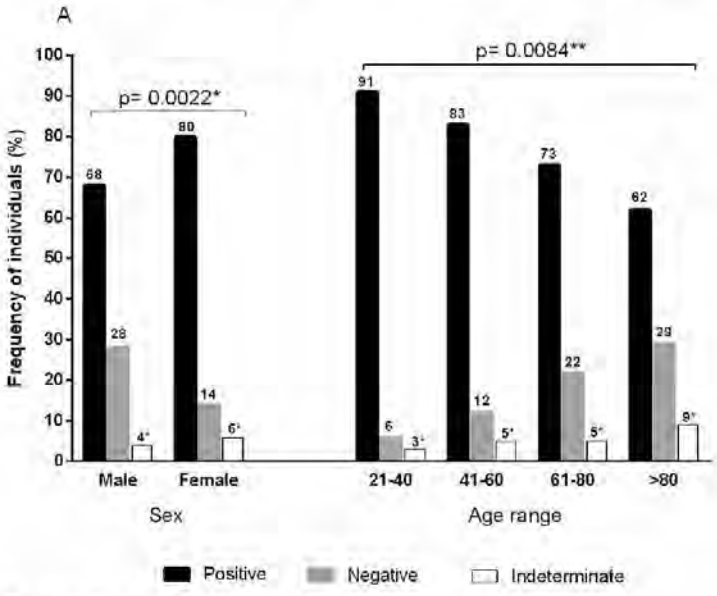


Figure 1. *Cont.*

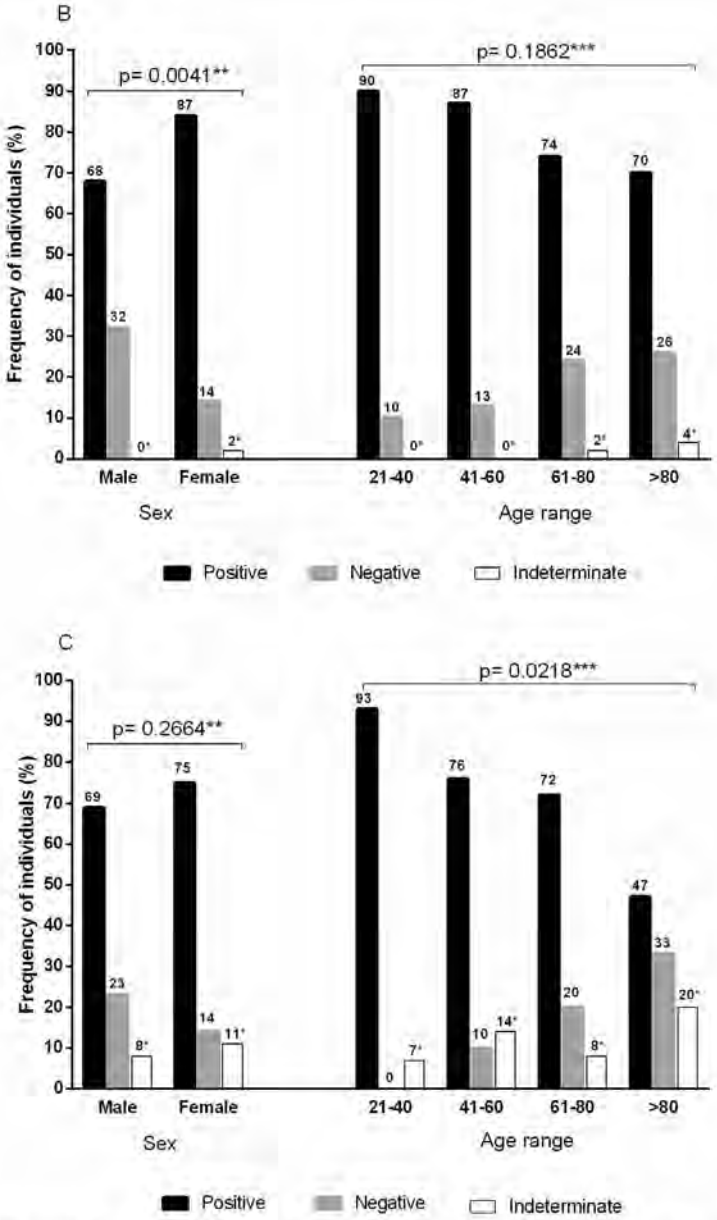


Figure 1. Frequencies of anti-SARS-CoV-2 antibodies according to sex and age group. (**A**) Pooled frequencies of anti-SARS-CoV-2 total antibodies (S1/S2) plus anti-SARS-CoV-2 IgG neutralizing antibodies (RBD-S1). Sample size by sex: male (n = 138) and female (n = 220). Sample size by age group: 21–40 (n = 35), 41–60 (n = 60), 61–80 (n = 221), and >80 (n = 42). (**B**) Frequencies of total anti-SARS-CoV-2 antibodies (S1/S2). Sample size by sex: male (n = 77) and female (n = 128). Sample size by age group: 21–40 (n = 20), 41–60 (n = 39), 61–80 (n = 119), and >80 (n = 27). (**C**) Frequencies of neutralizing IgG anti-SARS-CoV-2 (RBD-S1) antibodies. Sample size by sex: male (n = 61) and female (n = 92). Sample size by age group: 21–40 (n = 15), 41–60 (n = 21), 61–80 (n = 102), and >80 (n = 15). * Indeterminate results were not included in the statistical analysis; ** chi-square test; *** G test.

4. Discussion

The prevalence of seropositivity for anti-SARS-CoV-2 total antibodies and anti-SARS-CoV-2 neutralizing antibodies was evaluated in the present study in individuals vaccinated with two doses of Coronavac. The results were similar, regardless of the method used to assess humoral immunological response, including the frequency of those who did not produce antibodies. However, seropositivity values were lower than those reported by the manufacturer of the immunizing agent during phase I and II randomized clinical trials in adults, young people, and elderly people over 60 years, and were higher than the value of vaccine efficacy reported by health care professionals in direct contact with COVID-19 patients [17]. A limitation of the present study is the lack of information on the occurrence of previous infection in vaccinated individuals, a variable that might interfere with the assessment of post-vaccination seroconversion.

Recent studies have shown that the Coronavac vaccine is efficient in eliciting neutralizing antibodies [18–21], which together with the present results, particularly those obtained for anti-SARS-CoV-2 neutralizing antibodies, are encouraging. Indeed, considering the percentage of positivity observed in the present study, the findings suggest that mass vaccination of the population with Coronavac can generate collective protection [22].

Overall, there are different opinions and discussions about the efficacy and efficiency of immunizations with anti-SARS-CoV-2 vaccines in relation to the potential for generating protective immunity and the persistence of immunological memory [9,23], especially with regard to variables such as sex and age. In general, the immune response exhibits distinct dynamics based on factors such as sex [13,24] and immune system senescence [14]. In the present study, the frequency of anti-SARS-CoV-2 total antibodies was significantly higher in females, which corroborates the literature describing females as presenting increased inflammatory and humoral responses to COVID-19 [14], but we do not rule out the possibility that this result is due to a sampling bias.

Furthermore, with regard to seropositivity for anti-SARS-CoV-2 neutralizing antibodies, a high prevalence was observed among young adults; the lowest frequency was detected among elderly individuals, which suggests a lower effectiveness of the vaccine to stimulate the humoral immune response in the elderly. Despite the small sample size investigated herein, which can be a limitation of our study, our results seem to support evidence for a functional and progressive decline in the immune system in elderly patients [14]. Nevertheless, it is noteworthy that a recent phase I/II clinical trial study demonstrated immunogenicity after Coronavac vaccination in adults aged 60 years and older as well as its safety and tolerability [21]. It is important to emphasize that a lack of post-vaccination humoral immune response detection does not indicate the absence of immunity to SARS-CoV-2, as the serological methods used do not assess the presence of cellular immunity ($CD4^+$ and $CD8^+$ T lymphocytes), which may occur even in the absence of antibodies [25,26].

5. Conclusions

The results presented herein demonstrate a similar pattern in the frequency of seropositivity for anti-SARS-CoV-2 total antibodies and anti-SARS-CoV-2 neutralizing antibodies in individuals who received two doses of the Coronavac vaccine. However, a lower effectiveness of the humoral immune response among the elderly was found, which may be associated with senescence of the immune system. It is important that this result is confirmed in follow-up studies because a third-dose booster might be necessary for this group, especially due to their greater vulnerability to the most severe clinical outcome of COVID-19.

Finally, considering the emergence of virus variants, the neutralizing antibody response after vaccination should be monitored. Our results support the execution of population-based serological studies aimed at better understanding the efficacy of vaccines approved for use in Brazil in terms of their ability to generate herd immunity against SARS-CoV-2.

Author Contributions: Conceived the project, A.C.R.V. and C.D.A.B.; performed the laboratory analyses, C.D.A.B., I.P.C.d.A. and G.L.V.; performed the statistical analyses and wrote the draft of the article, C.D.A.B., E.d.S.G.A. and M.A.F.Q.; reviewed the article, A.C.R.V., R.I., C.N.C.B. and I.M.V.C.V. All authors have read and agreed to the published version of the manuscript.

Funding: This project was funded by the National Council for Scientific and Technological Development (CNPq), the Ministry of Science, Technology, and Innovation (MCTI), and the Ministry of Health (MS); Process CNPQ 401235/2020-3; Amaral Costa Medicina Diagnóstica and Federal University of Pará-Support Program for Qualified Production (PAPQ-2021). ACRV and RI are CNPq Grantees (#301869/2017-0 and #312979/2018-5).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Human Research Ethics Committee of the Institute of Health Sciences of the Federal University of Pará (CAAE: 31800720.1.0000.0018) in compliance with the guidelines and regulatory standards for research involving human beings. Individuals who agreed to participate in the study signed an informed consent form.

Informed Consent Statement: The study was approved by the Ethics and Research in Human Beings Committee of the Health Sciences Institute of the Federal University of Pará (CAAE: 31800720.1.0000.0018), and written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available upon request from the corresponding author.

Acknowledgments: We thank all individuals who agreed to participate in this study.

Conflicts of Interest: The authors declare no conflict of interest, and the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. World Health Organization. Coronavirus Disease (COVID-2019) Situation Reports. 2020. Available online: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/ (accessed on 27 March 2020).
2. Cheng, C.; Wang, H.Y.; Chan, L. Multiple forms of mass anxiety in coronavirus disease-2019 pandemic. *J. Affect. Disord.* **2021**, *291*, 338–343. [CrossRef]
3. Rubin, R. COVID-19 Vaccines vs. Variants-Determining How Much Immunity Is Enough. *JAMA* **2021**, *325*, 1241–1243. [CrossRef]
4. Corey, L.; Mascola, J.R.; Fauci, A.S.; Collins, F.S. A strategic approach to COVID-19 vaccine R&D. *Science* **2020**, *368*, 948–950. [CrossRef] [PubMed]
5. Park, J.H.; Lee, H.K. Delivery Routes for COVID-19 Vaccines. *Vaccines* **2021**, *9*, 524. [CrossRef] [PubMed]
6. Zhao, Q.; Gao, Y.; Xiao, M.; Huang, X.; Wu, X. Synthesis and immunological evaluation of synthetic peptide based anti-SARS-CoV-2 vaccine candidates. *Chem. Commun.* **2021**, *57*, 1474–1477. [CrossRef]
7. World Health Organization (WHO). COVID-19 Vaccine Tracker and Landscape. Available online: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines (accessed on 7 June 2021).
8. Fedele, F.; Aria, M.; Esposito, V.; Micillo, M.; Cecere, G.; Spano, M.; De Marco, G. COVID-19 vaccine hesitancy: A survey in a population highly compliant to common vaccinations. *Hum. Vaccin. Immunother.* **2021**, *7*, 1–7. [CrossRef]
9. Blumental, S.; Debré, P. Challenges and Issues of Anti-SARS-CoV-2 Vaccines. *Front. Med.* **2021**, *8*, 664179. [CrossRef]
10. Zhang, J.; He, Q.; An, C.; Mao, Q.; Gao, F.; Bian, L.; Wu, X.; Wang, Q.; Liu, P.; Song, L.; et al. Boosting with heterologous vaccines effectively improves protective immune responses of the inactivated SARS-CoV-2 vaccine. *Emerg. Microbes Infect.* **2021**, *10*, 1598–1608. [CrossRef]
11. Cerqueira-Silva, T.; de Araújo Oliveira, V.; Pescarini, J.; Bertoldo Júnior, J.; Mota Machado, T.; Flores-Ortiz, R.; Penna, G.; Ichihara, M.Y.; Barros, J.V.; Boaventura, V.S.; et al. Influence of age on the effectiveness and duration of protection in Vaxzevria and CoronaVac vaccines. *medRxiv* **2021**. [CrossRef]
12. ANVISA. Agencia Nacional de Vigilância Sanitárias. Vacinas—COVID-19. Available online: https://www.gov.br/anvisa/pt-br/assuntos/paf/coronavirus/vacinas (accessed on 7 June 2021).
13. Ulhaq, Z.S.; Soraya, G.V.; Zambrano, L.E.A.; Garcia, C.P. Sexual dimorphism in SARS-COV-2 infection. *Acta Endocrinol.* **2020**, *16*, 522–523. [CrossRef]
14. Ciarambino, T.; Para, O.; Giordano, M. Immune system and COVID-19 by sex differences and age. *Women's Health* **2021**, *17*, 17455065211022262. [CrossRef]
15. Edara, V.V.; Hudson, W.H.; Xie, X.; Ahmed, R.; Suthar, M.S. Neutralizing Antibodies against SARS-CoV-2 Variants after Infection and Vaccination. *JAMA* **2021**, *325*, 1896–1898. [CrossRef]
16. McDonald, J.H. G-test of goodness-of-fit. In *Handbook of Biological Statistics*, 3rd ed.; Sparky House Publishing: Baltimore, MD, USA, 2014; pp. 53–58.

17. Instituto Butantan. Vacina Adsorvida COVID-19 (Inativada). Available online: https://vacinacovid.butantan.gov.br/bulas (accessed on 8 June 2020).
18. Rogliani, P.; Chetta, A.; Cazzola, M.; Calzetta, L. SARS-CoV-2 Neutralizing Antibodies: A Network Meta-Analysis across Vaccines. *Vaccines* **2021**, *9*, 227. [CrossRef]
19. Tanriover, M.D.; Doğanay, H.L.; Akova, M.; Güner, H.R.; Azap, A.; Akhan, S.; Köse, Ş.; Erdinç, F.Ş.; Akalın, E.H.; Tabak, Ö.F.; et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): Interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *Lancet* **2021**, *398*, 213–222. [CrossRef]
20. Cao, Y.; Yisimayi, A.; Bai, Y.; Huang, W.; Li, X.; Zhang, Z.; Yuan, T.; An, R.; Wang, J.; Xiao, T.; et al. Humoral immune response to circulating SARS-CoV-2 variants elicited by inactivated and RBD-subunit vaccines. *Cell Res.* **2021**, *31*, 732–741. [CrossRef]
21. Wu, Z.; Hu, Y.; Xu, M.; Chen, Z.; Yang, W.; Jiang, Z.; Li, M.; Jin, H.; Cui, G.; Chen, P.; et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: A randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect. Dis.* **2021**, *21*, 803–812. [CrossRef]
22. Omer, S.B.; Yildirim, I.; Forman, H.P. Herd Immunity and Implications for SARS-CoV-2 Control. *JAMA* **2020**, *324*, 2095–2096. [CrossRef] [PubMed]
23. Widge, A.T.; Rouphael, N.G.; Jackson, L.A.; Anderson, E.J.; Roberts, P.C.; Makhene, M.; Chappell, J.D.; Denison, M.R.; Stevens, L.J.; Pruijssers, A.J.; et al. Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination. *N. Engl. J. Med.* **2021**, *384*, 80–82. [CrossRef] [PubMed]
24. Jin, S.; An, H.; Zhou, T.; Li, T.; Xie, M.; Chen, S.; Chen, C.; Ying, B.; Xu, Z.; Li, X.; et al. Sex- and age-specific clinical and immunological features of coronavirus disease 2019. *PLoS Pathog.* **2021**, *17*, e1009420. [CrossRef] [PubMed]
25. Grifoni, A.; Weiskopf, D.; Ramirez, S.I.; Mateus, J.; Dan, J.M.; Moderbacher, C.R.; Rawlings, S.A.; Sutherland, A.; Premkumar, L.; Jadi, R.S.; et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* **2020**, *181*, 1489–1501.e15. [CrossRef] [PubMed]
26. Gallais, F.; Velay, A.; Nazon, C.; Wendling, M.J.; Partisani, M.; Sibilia, J.; Candon, S.; Fafi-Kremer, S. Intrafamilial Exposure to SARS-CoV-2 Associated with Cellular Immune Response without Seroconversion, France. *Emerg. Infect. Dis.* **2021**, *27*, 113–121. [CrossRef] [PubMed]

1.13. CoronaVac produz anticorpos contra Covid-19 em 87% dos vacinados com duas doses na Indonésia

A CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, produziu anticorpos contra o SARS-CoV-2 em 87,15% dos imunizados pelo menos 28 dias após a segunda dose, segundo estudo realizado com milhares de pessoas na Indonésia.

Esse é o resultado do estudo clínico de fase 3 feito por cientistas da Faculdade de Medicina da Universitas Padjadjaran, em Bandung, e pelo Ministério da Saúde da Indonésia publicado na revista Vaccine em setembro de 2021.

O ensaio clínico randomizado, duplo-cego e controlado por placebo foi realizado em um total de 1.620 adultos saudáveis com idades entre 18 e 59 anos, divididos aleatoriamente entre os que receberam as duas doses ou placebo, entre os meses de agosto, setembro e outubro de 2020.

Para os que receberam as duas doses, a eficácia da CoronaVac foi de 65,30% - uma alta eficácia que segue o padrão demonstrado em estudos realizados com a vacina em outros países, como Turquia, Chile e Brasil.

CoronaVac evitou casos graves e mortes

Durante o período de vigilância do estudo, houve 49 casos de Covid-19 entre os voluntários. Destes, sete imunizados e 18 casos no grupo placebo foram sintomáticos e ocorreram entre um período de 14 dias a três meses após a segunda dose. Não houve relato de casos graves, críticos ou óbitos por Covid-19 entre os participantes do estudo.

Para a avaliação de segurança, os eventos adversos solicitados e não solicitados foram coletados após a primeira e segunda vacinação em 14 e 28 dias, respectivamente. Amostras de sangue foram coletadas para um ensaio de anticorpos antes e 14 dias após a segunda dose.

A maioria das reações adversas foram classificadas como leves e a mais relatada foi dor no local da injeção.

Dos 1.620 participantes, 1.046 eram do sexo masculino (64,57%) e 574 do sexo feminino (35,43%).

Publicado em: 10/10/2021

Artigo

Vaccine 39 (2021) 6520–6528

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

A phase III, observer-blind, randomized, placebo-controlled study of the efficacy, safety, and immunogenicity of SARS-CoV-2 inactivated vaccine in healthy adults aged 18–59 years: An interim analysis in Indonesia

Eddy Fadlyana [a,*], Kusnandi Rusmil [a], Rodman Tarigan [a], Andri Reza Rahmadi [a], Susantina Prodjosoewojo [a], Yulia Sofiatin [a], Citra V. Khrisna [a], Rini Mulia Sari [b], Lilis Setyaningsih [b], Fikrianti Surachman [b], Novilia Sjafri Bachtiar [b], Hadyana Sukandar [a], Imam Megantara [a], Chrysanti Murad [a], Krisna Nur A. Pangesti [c], Vivi Setiawaty [c], Sunarjati Sudigdoadi [a], Yaling Hu [d], Qiang Gao [d], Cissy B. Kartasasmita [a]

[a] Faculty of Medicine, Universitas Padjadjaran /Dr. Hasan Sadikin General Hospital, Bandung, Indonesia
[b] PT. Bio Farma, Bandung Indonesia
[c] National Institute of Health Research & Development, Jakarta, Indonesia
[d] Sinovac Life Sciences Co., Ltd., Beijing, China

ARTICLE INFO

Article history:
Received 21 May 2021
Received in revised form 8 September 2021
Accepted 21 September 2021
Available online 24 September 2021

Keywords:
Adults
Efficacy
Safety
Immunogenicity
SARS-CoV-2 Inactivated Vaccine

ABSTRACT

Background: The WHO declared COVID-19 a pandemic on March 11th, 2020. This serious outbreak and the precipitously increasing numbers of deaths worldwide necessitated the urgent need to develop an effective severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine. The development of COVID-19 vaccines has moved quickly. In this study, we assessed the efficacy, safety, and immunogenicity of an inactivated (SARS-CoV-2) vaccine.

Methods: We conducted a randomized, double-blind, placebo-controlled trial to evaluate the efficacy, immunogenicity, and safety of an inactivated SARS-CoV-2 vaccine and its lot-to-lot consistency. A total of 1620 healthy adults aged 18–59 years were randomly assigned to receive 2 injections of the trial vaccine or placebo on a day 0 and 14 schedule. This article was based on an interim report completed within 3 months following the last dose of study vaccine. The interim analysis includes safety and immunogenicity data for 540 participants in the immunogenicity subset and an efficacy analysis of the 1620 subjects. For the safety evaluation, solicited and unsolicited adverse events were collected after the first and second vaccination within 14 and 28 days, respectively. Blood samples were collected for an antibody assay before and 14 days following the second dose.

Results: Most of the adverse reactions were in the solicited category and were mild in severity. Pain at the injection site was the most frequently reported symptom. Antibody IgG titer determined by enzyme-linked immunosorbent assay was 97.48% for the seroconversion rate. Using a neutralization assay, the seroconversion rate was 87.15%. The efficacy in preventing symptomatic confirmed cases of COVID-19 occurring at least 14 days after the second dose of vaccine using an incidence rate was 65.30%.

Conclusions: From the 3-month interim analysis, the vaccine exhibited a 65.30% efficacy at preventing COVID-19 illness with favorable safety and immunogenicity profiles.

1. Introduction

The coronavirus disease 2019 (COVID-19) has inflicted catastrophic damage to public health, economic, and social stability worldwide [1]. In December 2019, a series of pneumonia cases of unknown origin emerged in Wuhan, Hubei, China, with clinical a presentation resembling viral pneumonia. The outbreak began in early November or December and the number of cases quickly

Abbreviations: COVID-19, Coronavirus Disease 2019; ELISA, Enzyme Link Immunoassay; GMT, Geometric Mean Titer; IgG, Immunoglobulin G; rRT-PCR, Real-time Reverse Transcriptase-PCR; SARS, Severe Acute Respiratory Syndrome; WHO, World Health Organization.

* Corresponding author.
E-mail addresses: eddy.fadlyana@unpad.ac.id (E. Fadlyana), y.sofiatin@unpad.ac.id (Y. Sofiatin), rini.mulia@biofarma.co.id (R.M. Sari), lilis.setyaningsih@biofarma.co.id (L. Setyaningsih), fikrianti.surachman@biofarma.co.id (F. Surachman), novilia@biofarma.co.id (N.S. Bachtiar), imam.megantara@unpad.ac.id (I. Megantara), huyl@sinovac.com (Y. Hu), gaoq@sinovac.com (Q. Gao).

https://doi.org/10.1016/j.vaccine.2021.09.052
0264-410X/

rose. As of May 2020, >80,000 cases were confirmed in China, including healthcare workers, which resulted in>4,000 deaths [2–5]. The virus is airborne, highly transmissible between humans, and has a long and insidious incubation period. The outbreak rapidly escalated out of China and throughout the world, pushing the World Health Organization (WHO) to declare a pandemic on March 11th, 2020 [6]. As of December 20th, 2020, the number of COVID-19 cases was>75 million with over 1.6 million deaths occurring globally [7]. Based on a WHO report, by January 20th, 2021, there were 939,948 confirmed cases of COVID-19 with 26,857 deaths in Indonesia [8].

Currently, there is no effective treatment available for coronavirus infection. Vaccination is crucial for blocking the rapid spread of deadly infectious diseases, such as the highly contagious COVID-19, especially when effective treatments or cures are not available [9]. Significant efforts have been focused on the development of vaccines and therapeutic drugs. Over the past decade, the scientific community and the vaccine industry have been asked to respond urgently to epidemics including H1N1 influenza, Ebola, Zika, and most recently, SARS-CoV-2 [10]. The WHO is currently preparing a comprehensive analysis of vaccine and therapeutic drug candidates that may be effective against SARS-CoV-2 and will use an evidence-based framework to transparently select the most promising therapeutic and vaccine candidates to evaluate in the clinic [11]. Multiple SARS-CoV-2 vaccines types, such as DNA-based and RNA-based formulations, recombinant subunit-containing viral epitopes, adenovirus-based vectors, and purified inactivated virus are under development. Purified inactivated viruses have been traditionally used for vaccine development and have been found to be safe and effective for preventing many viral diseases including influenza and polio [12–14].

As of January 25th, 2021, there are 64 vaccines in human clinical trials and 20 have reached the final stages of testing. At least 173 preclinical vaccines are under active investigation in animals [15].The preclinical study results of inactivated SARS-CoV-2 Vaccine (Vero Cell), developed by Sinovac Life Sciences Co. Ltd. indicate that the vaccine provided partial or complete protection in macaques from severe interstitial pneumonia after a SARS-CoV-2 challenge without observable antibody dependent enhancement [16]. A phase I/II clinical trial has been conducted in China since April 2020. The preliminary results indicate a favorable safety and immunogenicity profile with a two-dose vaccine schedule. No significant changes in inflammatory factors were observed indicating a small risk of immunopathology induced by the SARS-CoV-2 vaccine [17].

In this article, we report the efficacy of inactivated SARS-CoV-2 vaccine in preventing COVID-19 including safety and immunogenicity data based on the phase III trial collected during a 3-month period after the second injection in 18–59 year-old subjects in Indonesia. This data set and trial results form the basis of an application for emergency use authorization in Indonesia.

2. Materials and methods

2.1. Study design and population

This study was an observer-blinded, randomized, placebo-controlled two arm with parallel groups, prospective intervention, phase III study that began in August 2020 in Bandung, Indonesia to evaluate the efficacy, immunogenicity, and safety of an inactivated SARS-CoV-2 vaccine and its lot-to-lot consistency. The main exclusion criteria included evolving mild, moderate, or severe illness, especially infectious disease or fever (body temperature $\geq$ 37.5℃) , patients with serious chronic diseases, positive result from a nasopharyngeal swab RT-PCR test, reactive IgG and IgM for SARS-CoV-2, women who are lactating, pregnant or planning to become pregnant during the study period, serious chronic diseases (serious cardiovascular disease, uncontrolled hypertension and diabetes, liver and kidney disease, malignant tumors, or any condition which according to the investigator may interfere with the assessment of the trial objectives), uncontrolled coagulopathy or blood disorders, history of asthma, history of allergy to vaccines or vaccine ingredients, history of confirmed or suspected immunosuppressive or immunodeficient state, or received in the previous 4 weeks a treatment likely to alter the immune response [intravenous immunoglobulins, blood-derived products, or long-term corticosteroid therapy (>2 weeks)], history of uncontrolled epilepsy or other progressive neurological disorders, and having received any vaccination within 1 month before or after administration of the study vaccine.

After being informed about the study and signing an informed consent form, the medical history of the subjects was evaluated, and they were provided a physical exam. The blinded investigator team evaluated the inclusion and exclusion criteria. Eligible subjects were randomly assigned at a ratio of 1:1 into two study arms to receive either 3 μg/0.5 mL dose of inactivated SARS-CoV-2 vaccine or placebo on day 0 and 14. The randomization list was generated automatically using the website, www.sealedenvelope.com, and the vaccinated arms were grouped into three different batch numbers (batch 1/batch 2/batch 3) of SARS-CoV-2 vaccine. The subjects were randomized and vaccinated per treatment group by an unblinded team. The alphabetical code remained confidential and maintained by the unblinded team and was not to be opened until the end of the study.

The study protocol, subject information sheet and consent forms, and the subject's diary card was approved by the Research Ethics Committee of Universitas Padjadjaran (Ethical Approval No. 669/UN6.KEP/EC/2020) and Indonesian Regulatory Authorities. This trial was conducted in accordance with ICH Good Clinical Practice guidelines, the Declaration of Helsinki, and local regulatory requirements. The clinical trial was registered at clinicaltrials.gov with entry number NCT04508075 and in the Indonesian Clinical Research Registry (INA-WXFM0YX).

2.2. Study vaccine

The study vaccine, developed by Sinovac Life Sciences Co., Ltd., was an inactivated SARS-CoV-2 whole virion vaccine with aluminum hydroxide as an adjuvant. The study vaccine was manufactured by inoculating novel coronavirus (CZ02 Strain) into African green monkey kidney cells (Vero Cell). The virus was successfully incubated, harvested, inactivated using β-propiolactone, concentrated, purified, and adsorbed by aluminum hydroxide. The bulk vaccine was then formulated with phosphate-buffered saline and sodium chloride as the inactivated final product. A dosage of 3 μg/0.5 mL was selected for this study. Three batches of study vaccine were used (20200308, 20200412, and 20200419). The placebo contained water for injection packaged in ampoules (0.5 mL/dose) and manufactured by PT Bio Farma. The study vaccine was administered intramuscularly into the left deltoid region by an unblinded investigator. The vaccine was stored at + 2℃ to + 8℃.

2.3. Surveillance for COVID-19 and efficacy assessment

The primary outcome of the study was to assess the efficacy of two doses of the inactivated SARS-CoV-2 vaccine in preventing COVID-19 cases compared with placebo. The primary efficacy endpoint was incidence of laboratory confirmed-symptomatic COVID-19 cases starting at 14 days following the second dose. COVID-19 case defined according to the case definition of the national guidelines for the diagnosis and treatment of COVID-19

in Indonesia [18]. Subjects were surveilled for COVID-19 disease after the first dose of vaccine by a combination of active and passive surveillance. The surveillance team performed monthly contact (by phone or text message) to actively collect information from subjects whether they have any symptoms suggesting COVID-19 disease or admitted to hospital for any reason. Any subject who has at least one specific symptoms (cough, taste or smell disorders, or dyspnea) or has two or more non-specific symptoms (fever, chills, sore throat, fatigue, nasal congestion or runny nose, body pain, muscle pain, headache, nausea, vomiting, or diarrhea) for at least two consecutive days was scheduled to have nasopharyngeal swab sample taken for SARS-CoV-2 rRT-PCR test. Subjects were also regularly reminded to report if they have any of the above symptoms.

The rRT-PCR was performed by the Central Laboratory of Universitas Padjadjaran. Nasopharyngeal samples were processed in a dedicated BSL-2 laboratory with BSL-3 practices under a certified Class II Biological Safety Cabinet. Once a clinical sample was treated with lysis buffer for RNA extraction, the samples then moved to a less restrictive environment to complete the RNA extraction and real-time RT-PCR. A 140 µl aliquot of the specimen was added to 560 µl of lysis buffer (Qiagen Viral Mini kit). RNA extraction was done based on the manufacturer's protocol and immediately processed for RT-PCR. The remaining nucleic acid was stored at −80℃ for sequence analysis.

The real-time reverse transcriptase-PCR (rRT-PCR) reagent kit from ABT (Beijing Applied Bioscience Technology) and the Multiple Real-Time PCR Kit for Detection of 2019-nCoV were used. The results were analyzed by software provided by the manufacturer of the Light Cycler (Roche). Comparative viral load was calculated using the CT (Cycle Threshold) values of consecutive specimens. The incidence of suspected COVID-19 cases within 14 days to 6 months after the second dose of immunization was analyzed to determine efficacy.

2.4. Immunogenicity assessment

To assess the immune response, 4 mL blood samples were collected from 540 subjects before the first injection (Day 0) and 14 days after the second injection. The ability of the antibodies present in the blood sample to bind to the receptor binding domain (RBD) of SARS-CoV-2 was assessed blindly using an enzyme-linked immunosorbent assay (ELISA) at the Clinical Trial Laboratory of Bio Farma. The ELISA titers were determined by end point dilution and calculated using GraphPad Prism version 8.4.3 software [19–21]. The antibody increment and GMT 14 days post-last immunization were evaluated. ELISA seropositive antibody IgG titer was defined as titer > 200 and seroconversion was defined as a four-fold increase of anti-RBD antibody IgG titer (ELISA) at 14 days after two doses of vaccine compared with the baseline. The neutralization of antibody (NAb) assay was also conducted at the National Intitute of Health Reasearch & Development. A four-fold increase in antibody titer compared with the baseline value was considered as the measure of seroconversion. Seropositivity was defined as detected antibody $\geq$ 1:4. The immunogenicity data were analyzed in the per protocol population using SPSS software. Pre-vaccination titer levels for subjects with zero titer were assigned a value of 200 for ELISA and 2 to enable GMT and titer increment calculations.

2.5. Safety assessment

Subjects were given diary cards to record solicited adverse events (local pain, redness, swelling, induration, fever, myalgia, and malaise) and unsolicited adverse events occurring within 30 min, 7 days, and 8–28 days following each dose. Pain was graded as mild (pain at injection site when touched), moderate (pain with movements), and severe (significant pain at rest). Redness, induration, and swelling intensity were measured using a plastic bangle and categorized as mild (<5 cm), moderate (5–10 cm), and severe (>10 cm). Fever was graded as mild (38.0–38.4°C), moderate (38.5–38.9°C), and severe ($\geq$39.0°C). Fatigue, myalgia, and unsolicited events were graded as mild (no interference with activity), moderate (some interference with activity not requiring medical intervention), and severe (prevents daily activity, requires medical intervention).

Any serious adverse events were reported up to 6 months after the second dose. Diary card was reviewed by the blinded investigator at 14 days following the first injection, 14, and 28 days after the second injection. The safety data were reviewed by a Data Safety Monitoring Board (DSMB) and analyzed in the intention-to-treat population using SPSS software.

2.6. Sample size determination and statistical analysis

The study was powered for efficacy analysis. Sample size was determined based on 95% confidence interval and 80% power. Assuming that 2% of the population would develop COVID-19 infection in the placebo arm, a minimum of 810 subjects in each vaccinated and placebo group would provide 80% power to reject the null hypothesis of no difference if the true efficacy was 60% with a 5% dropout rate. In this study, the total cohort was 1620 subjects with 810 subjects in the vaccinated group and 810 subjects in the placebo group.

Vaccine efficacy (VE) will be estimated by (1 - RR) $\times$ 100, where RR (relative risk) is calculated as the incidence in the vaccinated group divided by the incidence in the placebo group per person-years.

To analyze the immunogenicity, GMTs comparation between vaccine and placebo group was calculated after logarithmic transformation using *t*-test or ANOVA (F-test). Serum immune response proportions (seropositive rate, seroconversion) and vaccine lot-to-lot comparison was calculated using Chi-square test. The incidence rates of solicited and unsolicited adverse events between both groups were analyzed using Chi-square test. A p-value of<0.05 was considered to be significant.

3. Results

3.1. Study population

Between August 11, 2020, and October 21, 2020, a total of 1819 participants were screened and 199 subjects were excluded due to not meeting the inclusion criteria or meeting one of the exclusion criteria. From 1620 subjects randomized in the study, there were 17 subjects that withdrawn from the study prior to the second dose [Fig. 1]. The first 540 participants were included in the immunogenicity subset group.

There were 1046 male participants (64.57%) and 574 female participants (35.43%). The participants were come from various age distribution from 18 to 59 years with average 35.5 ± 11.2 years old. Among the subset immunogenicity subjects, there were 314 male participants (58.15%) and 226 female participants (41.85%) with an average age of 35.82 years ± 11.4 years old. The details of the demographic data are provided in Table 1.

All study vaccines were administered according to the randomization list. Treatment compliance was defined as receiving both doses of vaccine/placebo within the specified time period. For the 540 participants in the immunogenicity subset, 10 subjects withdrew prior to the second dose vaccination and not included in the immunogenicity analysis. Meanwhile, 1 subject withdrew after

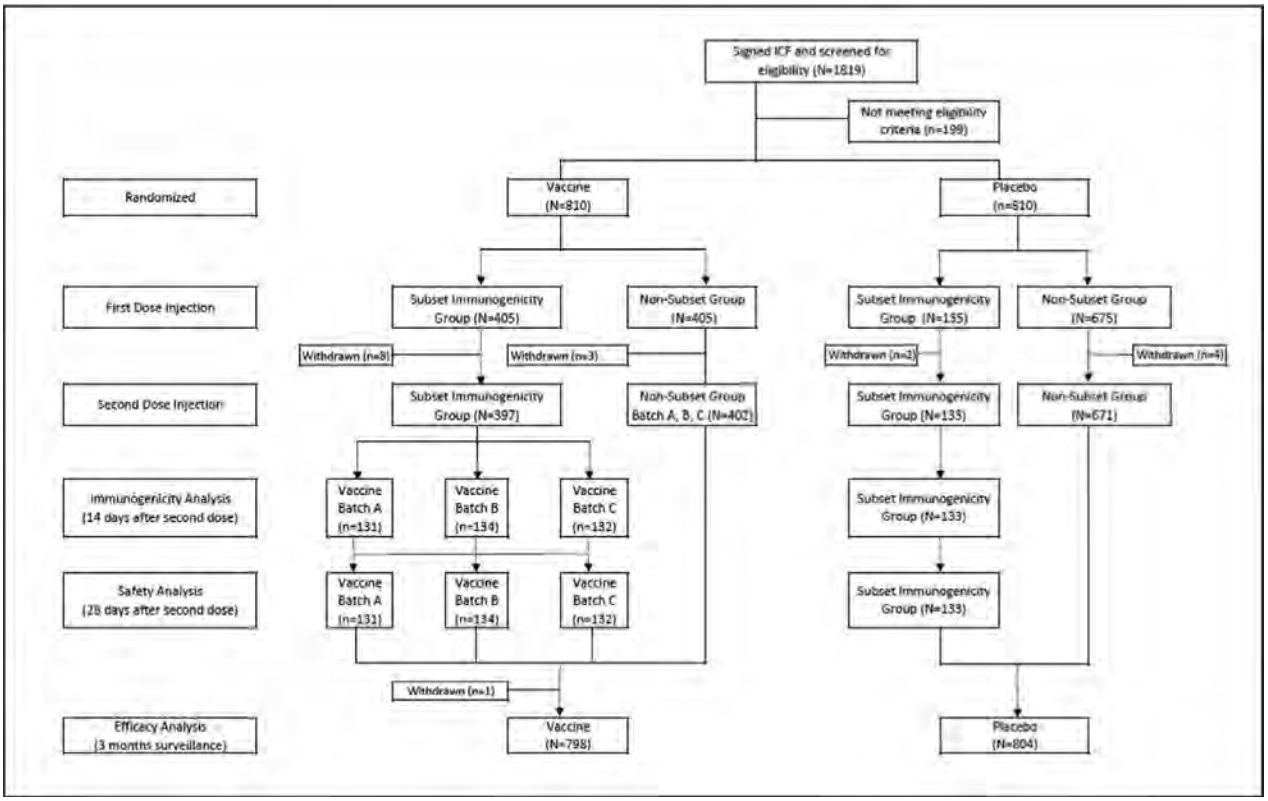


Fig. 1. Participant Disposition.

Table 1
Demographic Data.

Parameter	Vaccine (N = 811)	Placebo (N = 809)	Total (N = 1620)
Mean age [years] (SD)	35.6 (11.3)	35.4 (11.0)	35.5 (11.2)
Mean height [m] (SD)	1.63 (0.09)	1.63 (0.09)	1.63 (0.09)
Mean weight [kg] (SD)	65.6 (13.5)	64.8 (13.6)	65.2 (13.5)
BMI (kg/m^2)	24.8 (4.4)	24.5 (4.5)	24.6 (4.5)
Sex n(%)			
Male	505 (62.3)	541 (66.8)	1046 (64.57)
Female	305 (37.7)	269 (33.2)	574 (35.43)
Demographic Data in the Immunogenicity Subset Group			
Parameter	Vaccine (N = 405)	Placebo (N = 135)	Total (N = 540)
Mean age [years] (SD)	36.0 (11.5)	35.3 (10.9)	35.82 (11.4)
Mean height [m] (SD)	161.8 (8.9)	161.7 (9.8)	161.8 (9.2)
Mean weight [kg] (SD)	64.6 (13.2)	65.9 (13.6)	64.9 (13.3)
BMI (kg/m2)	24.6 (4.3)	25.2 (4.7)	24.75 (4.4)
Sex n(%)			
Male	229 (56.5)	85 (63.0)	314 (58.15)
Female	176 (43.5)	50 (37.0)	226 (41.85)

Abbreviations: N = number of participants, SD = Standard deviation.

Table 2
Treatment Compliance in Immunogenicity Subset Group.

	Vaccine n (%)	Placebo n (%)	Total N (%)
Subjects screened for RT-PCR test	405	135	540
Subjects screened for IgM/IgG test	405	135	540
Subjects enrolled	405	135	540
First vaccination completed	405	135	540
Second vaccination completed	397	133	530
Intention-to-treat population (for safety and efficacy analysis)	405	135	540
Per-protocol population (for immunogenicity analysis 14 days after last injection)	397	133	530

the second dose of the study vaccine. These dropout subjects included 9 from the vaccinated group and 2 from the placebo group. The details for treatment compliance in the subset immunogenicity group are presented in Table 2. Early withdrawal resulted from consent withdrawal by the subject or the subject met the contraindication criteria for the second vaccination (not in healthy condition during the second vaccination schedule). The study results presented in this article are based on a preliminary immunogenicity and safety data analysis of 540 subjects in the immunogenicity subset group, whereas the efficacy results are based on preliminary efficacy data from 1620 subjects with median ~ 2.5 months of surveillance period.

3.2. Efficacy

During the surveillance period, 320 COVID-19 suspect cases and 49 laboratory confirmed COVID-19 cases were collected. From these 49 confirmed COVID-19 cases, 25 cases (7 cases in the vaccine group and 18 cases in the placebo group) were symptomatic and occurred from 14 days following the second dose up to 3 months. There were no severe, critical, or deaths of laboratory confirmed COVID-19 cases observed [Table 3].

Vaccine efficacy was defined as percentage reduction in relative risk using the ratio of incidence rate in the vaccine group and placebo group. Incidence rate was calculated by the number of subjects with laboratory-confirmed COVID-19 divided by the total

Table 3
Summary of Primary Efficacy Endpoint.

	Vaccine			Placebo			
Endpoint	No. of cases	Mean follow-up days	Incidence rate (per 100 person years)	No. of cases	Mean follow-up days	Incidence rate (per 100person years)	Vaccine Efficacy (%)
Symptomatic confirmed laboratory cases COVID-19 starting 14 days after second injection	7	80.78		18	72.08		65.30%
			3.904			11.25	
Severe	0		0	0		0	
Critical	0		0	0		0	–
Death	0		0	0		0	

number of subjects at risk adjusted by time (person years). The vaccine showed 65.3% efficacy in preventing symptomatic COVID-19.

3.3. Immunogenicity

3.3.1. Antibody IgG titer by ELISA

The seropositive rate of SARS-CoV-2 IgG antibody in the vaccine group at 14 days after the second injection was 99.74%. The seropositive rate in the vaccine group increased significantly compared with the placebo group. The seroconversion rate at 14 days after the second injection in the vaccine group was 97.48% which was significantly different compared with a 0.75% seroconversion rate in the placebo group. There was a 23.5-fold increase of IgG antibody GMT at 14 days after the second injection in the vaccine group, whereas there was no significant increase of GMT in the placebo group. The results of the IgG analysis using ELISA are presented in Table 4.

3.3.2. Neutralization antibody

Neutralization antibody seropositive was defined as a titer $\geq$ 1:4 and seroconversion was defined as a change from a titer < 1:8 to a titer $\geq$ 1:8; or a 4-fold increase from baseline if the titer at baseline $\geq$ 1:8. After the full schedule of vaccine administration, the seropositive rate of SARS-CoV-2 antibody using the neutralization assay in the vaccine group at 14 days was significantly different compared with that of the placebo group. The seroconversion rate 14 days after the second injection in the vaccine group was 87.15% with no seroconversion in the placebo group. There was a 7.88-fold increase of antibody neutralization GMT at 14 days after the second injection. The neutralization antibody results are presented in Table 4.

3.3.3. Lot-to-lot consistency

Another objective of the study was to evaluate the consistency of 3 batches of inactivated SARS-CoV-2 vaccine. The IgG antibody seropositive rate for the three batches of vaccine (batch numbers 20200308, 20200412, and 20200419) were 100%, 99.25%, and 100%, respectively, whereas the seroconversion rates were 96.18%, 97.76%, and 98.48%, respectively for the 14 day time point after the second vaccination. The GMT of the three batches was 5093.78, 5421.63, and 5032.34, respectively, for the 14 day time point after the second injection.

Table 4
Antibody Titer between the Vaccine and Placebo Groups.

Antibody Titer	Time Point	Parameter	Group		p-value
			Vaccine (N = 397)	Placebo (N = 133)	
IgG (ELISA)	**V1**	Seropositive rate n(%)	44 (11.08)	14 (10.53)	0.859**
		(95% CI)	(8.36–14.55)	(6.37–16.89)	
		GMT*	220.27	220.37	0.990***
		(95% CI) Median	(212.87–227.93)	(206.45–235.24)	
			200.00	200.00	
	V3	Seropositive rate n(%)	396 (99.74)	7 (5.29)	<0.001**
		(95 %CI)	(99.26–100)	(1.47–9.06)	< 0.001**
		Seroconversion n(%)	387 (97.48)	1 (0.75)	< 0.001***
		(95% CI)	(95.43–98.63) 5181.19	(0.13–4.14)	
		GMT* (95% CI) Median	(4746.13–5656.14)	223.61	
			5333.35	(209.08–239.47)	
				200.00	
Neutralization Antibody	**V1**	Seropositive rate n(%) (95% CI)	0 (0–0.96)	0 (0–2.81)	–
		GMT* (95% CI) Median	2.00 (–)	2.00 (–)	
			–	–	–
	V3	Seropositive rate n(%)	380 (95.72)	1 (0.75)	<0.001**
		(95% CI)	(93.25–97.31)	(0.13–4.14)	< 0.001**
		Seroconversion n (%) (95% CI)	346 (87.15)	0 (0.00)	< 0.001***
		GMT* (95% CI)	(83.50–90.09)	(0–2.81)	
		Median	15.76	2.02	
			(14.57–17.04)	(1.98–2.05)	
			16	2	

*) The comparison results after logarithmic transformation. **) Chi-square test; ***) *t*-test.
V1 = before injection;
V3 = 14 days after second injection;
IgG seropositive = titer > 200; seroconversion = four-fold increasing anti-RBD antibody IgG titer compare to baseline 14 days after the second dose.
Antibody neutralization seropositive = titer $\geq$ 1:4; seroconversion = a change from seronegative (titer < 1:8) to seropositive (titer $\geq$ 1:8); or a 4-fold increase from baseline titers if titer at baseline $\geq$ 1:8.

We compared the proportion of participants with seropositive and seroconversion between the 3 batches of SARS-CoV-2 vaccine. The results indicated that there was no significantly different proportion between the 3 vaccine batches as shown in Table 5.

After the full schedule of vaccine, the seropositive rate of SARS-CoV-2 antibody as determined by the neutralization assay for batch numbers 20200308, 20200412, and 20,200,419 at 14 days after the second injection was above 94%. The seroconversion rate for each vaccine batch at 14 days after the second injection was 90.08%, 88.81%, and 82.58%, respectively. There was an increase of 7 to 8-fold for neutralization antibody GMT in all batches at 14 days following the second injection.

3.4. Safety

Within the immunogenicity subset group (n = 540), the majority of the reported local reactions was local pain, whereas the most common systemic event was myalgia. In the vaccine group, local pain was reported by 33.5% and 30.5% of the subjects after the first and second injection, respectively [Fig. 2]. In the placebo group, local pain was reported by 23.7% and 30.1% of the subjects after the first and second injection, respectively. In the vaccine group, myalgia was reported by 25.6% and 19.9% of the subjects after the first and second injection, respectively. In the placebo group, myalgia was reported by 12.6% and 9.0% of the subjects after the first and second injection, respectively. Based on the system organ class, majority of the unsolicited adverse event was categorized in the nervous system diseases category, specifically headache [Table S1].

The intensity of the adverse events was mostly mild in the vaccine and placebo groups. After the first injection, the percentage of mild adverse events in the vaccine and placebo groups was 54.3% and 46.7%, respectively. After the second injection, the percentage of mild adverse events in the vaccine and placebo groups were 47.9% and 42.9%, respectively. There was a significant difference in the distribution of severe adverse reactions after the second dose between the vaccine and placebo groups, with a higher proportion in the placebo group. Moderate adverse reactions after the first dose in the vaccine groups were significantly higher than the placebo group.

Of the 1620 subjects enrolled to the study, there were nine serious adverse events (SAE) that occurred in all subjects with a classification not related to vaccine products (five SAEs). One SAE was very unlikely and three SAEs were reported as less likely to be related to the vaccine product as assessed by the DSMB.

4. Discussion

The efficacy of 2 doses of SARS-CoV-2 vaccine at preventing COVID-19 was evaluated up to 6 months after the second dose of injection. However, this interim report consisted of an efficacy analysis of 1620 participants within 3 months following the final dose of study vaccine. The efficacy analysis was performed based on the primary endpoint for all enrolled subjects with a data cut-off date of January 9th, 2021. The efficacy in preventing symptomatic confirmed cases of COVID-19 occurring at least 14 days after the second dose of vaccine was 65.30% (person years) with 7 COVID-19 cases occurring in the vaccine group and 18 COVID-19 cases occurring in the placebo group. There were no severe, critical, or incidents of death from laboratory confirmed COVID-19 infection.

Table 5
Comparison of Antibody Titer in Different Vaccine Batches.

Antibody	Time Point	Parameter	Batch			p-value**
			Batch 20200308 (n = 131)	Batch 20200412 (n = 134)	Batch 20200419 (n = 132)	
IgG (ELISA)	**V1**	Seropositive rate n(%)	14 (10.70)	16 (11.94)	14 (10.61)	0.927**
		(95% CI)	(6.47–17.14)	(7.48–18.52)	(6.42–17.02)	
		GMT*)	215.16	223.40	222.26	0.384***
		(95% CI)	(205.70–225.05)	(208.36–239.52)	(209.08–236.27)	
		Median	200.00	200.00	200.00	
	V3	Seropositive rate n (%)	131 (100)	133 (99.25)	132 (100)	0.374**
		(95% CI)	(97.15–100)	(95.89–99.87)	(97.17–100)	
		Seroconversion n (%)	126 (96.18)	131 (97.76)	130 (98.48)	0.476**
		(95% CI)	(92.38–98.36)	(93.62–99.24)	(94.64–99.58)	
		GMT*)	5093.78	5421.63	5032.34	
		(95% CI)	(4369.78–5937.59)	(4656.29–6312.77)	(4314.30–5869.76)	0.898***
		Median	5105.05	5787.62	5302.40	
Neutralization Antibody	**V1**	Seropositive rate n(%)	0	0	0	–
		(95% CI)	(0–2.85)	(0–2.94)	(0–2.91)	
		GMT*)	2.00	2.00	2.00	–
		(95% CI)	–	–	–	
		Median	–	–	–	
	V3	Seropositive rate n (%)	126 (96.18)	127 (94.78)	127 (96.21)	0.803**
		(95% CI)	(91.38–98.36)	(89.61–97.45)	(91.44–98.37)	
		Seroconversion n (%)	118 (90.08)	119 (88.81)	109 (82.58)	0.150**
		(95% CI)	(83.76–94.11)	(82.35–93.10)	(75.21–88.10)	
		GMT*)	15.97	16.59	14.75	0.470***
		(95% CI)	(14.03–18.18)	(14.47–19.02)	(12.78–17.02)	
		Median	16.00	16.00	16.00	

*) The comparison results after logarithmic transformation. **) Chi-square test; ***) ANOVA (F-test).
V1 = before injection.
V3 = 14 days after second injection.
IgG seropositive = titer > 200; seroconversion = four-fold increasing anti-RBD antibody IgG titer compare to baseline 14 days after the second dose.
Antibody neutralization seropositive = titer ≥ 1:4; seroconversion = a change from titer < 1:8 to titer ≥ 1:8; or a 4-fold increase from baseline titers if titer ≥ 1:8 14 days after the second dose.

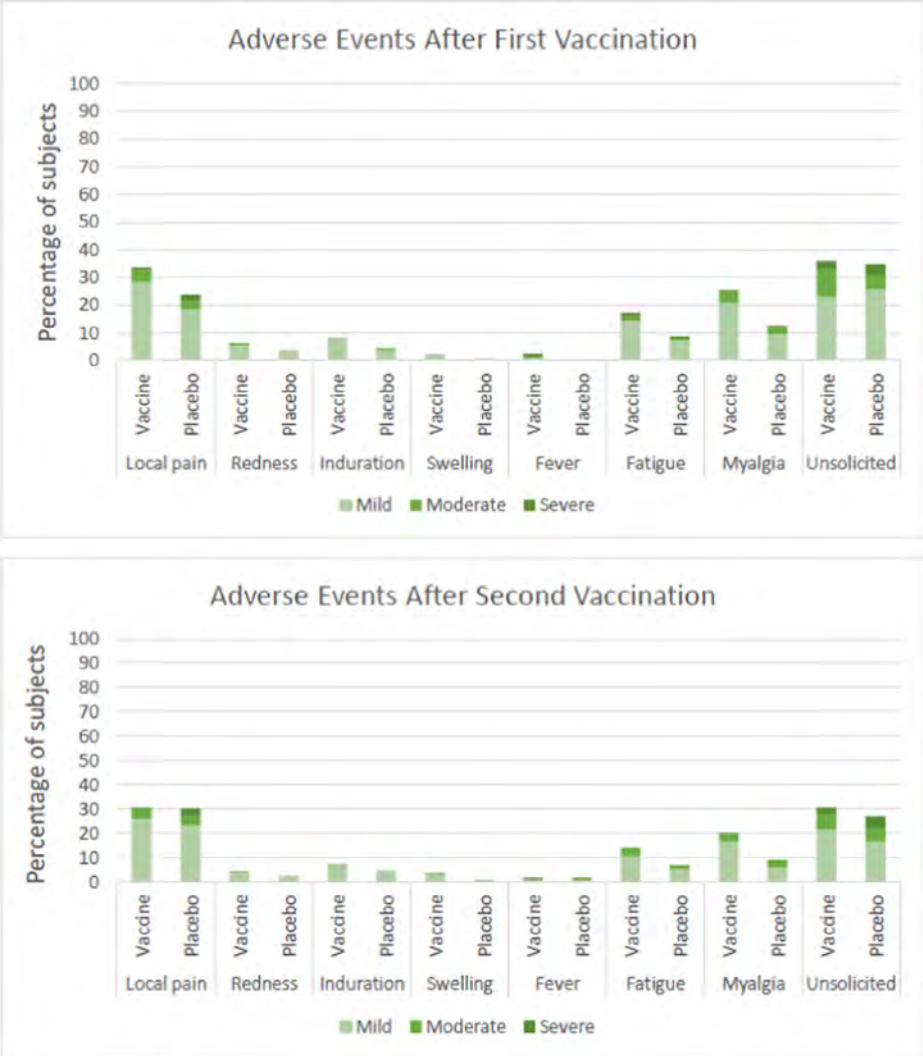


Fig. 2. Adverse Events occurring after the First and Second Vaccine Injection.

A phase III study for the study vaccine was also conducted in Brazil, Turkey, and Chile. Each country has a specific study design depending on its pandemic situation, but the main design is similar. Efficacy data from other countries may support the registration in each country. Based on the interim result, vaccine efficacy in Brazil and Turkey was 50.65% and 83.5%, respectively [22,23]. Vaccine effectiveness study was conducted in Chile with result of 65.9% [24]. The variability of efficacy result between the countries may reflect variance in study characteristics such as population, testing rate/capture of milder case, and force of infection [22].

The efficacy results in this study were higher compared with that of the same study in Brazil. The Brazilian study showed that after 14 days following vaccination with 2 doses of vaccine using a 0 and 14 day schedule, the efficacy rate against COVID-19 was 50.65% for all cases, 83.70% for cases requiring medical treatment, and 100.00% for hospitalized, severe, and fatal cases. This may be the result of Brazil having a high-risk population, particularly health care workers, thus leading to a higher COVID-19 infection rate. In contrast, the Indonesian study used the general population with a smaller occupational exposure to COVID-19 infection [22,25].

Efficacy is one of the key indices to evaluate a vaccine. It measures the effect of vaccination by calculating the proportionate reduction in cases among vaccinated subjects in a double-blind placebo-controlled randomized clinical trial. VE is measured by calculating the risk of disease among vaccinated and unvaccinated subjects and determining the percent reduction in risk of disease relative to the unvaccinated group. The greater the percent reduction of illness in the vaccinated group, the higher the VE [26–28].

In this study, the most common adverse events were pain at the site of injection and myalgia which were reported in vaccine and placebo recipients and with a significantly higher proportion of participants in the vaccinated group compared with the placebo group. Most adverse events were mild or moderate in severity. In the vaccine group, fever was reported in 2.5% of the participants after the first dose and 1.8% after the second dose of vaccine. No significant differences in proportion between the vaccine and placebo group were observed. Overall, reactogenicity events were mild and resolved within a couple of days after onset. These results indicate that the vaccine was well-tolerated. The occurrence of fever following vaccination with SARS-CoV-2 inactivated vaccine was lower compared with other COVID-19 vaccine candidates, such as the novel chimpanzee adenovirus vector vaccine, ChAdOx1 nCoV-19 viral-vector vaccines (18% in participants without paracetamol), or RNA vaccines (16% in younger vaccine recipients and by 11% of older recipients reported after the second dose) [29,30].

The immune response based on the seropositive and seroconversion rate of SARS-CoV-2 antibody IgG titer using ELISA at 14 days after the second injection were 99.74% and 97.48%, respectively. The IgG antibody GMT before injection and 14 days after the second injection were 220.27 and 5181.19, respectively. The seroconversion rate of RBD-specific IgG in this study were similar to that of the phase II study which was 97% [GMT 1094.3 (95% CI 936.7–1278.4)] at 14 days following the second dose [17].

The immune response based on the seropositive and seroconversion rate of SARS-CoV-2 neutralizing antibody using the neutralization assay in the vaccine group at 14 days after the second injection were 95.72% and 87.15%, respectively. The neutralization antibody GMT was 15.76 at 14 days after the second injection. The study vaccine phase I/II clinical trials conducted in China in April 2020 to evaluate the safety and immunogenicity of 2 doses of vaccine at intervals of 0 and 14 days (emergency schedule) and 0–28 days (routine schedule). In the phase I/II trials, it was found that immune responses induced by the day 0 and 28 vaccination schedule were larger than those induced from the day 0 and 14 vaccination schedule. In the phase 2 trial, the seroconversion rate of neutralizing antibodies to live SARS-CoV-2 for the same dosage used in this study were 92% with a GMT of 27.6 (95% CI 22.7–33.50) at 14 days after the second dose and 94% with a GMT of 23.8 (95% CI 20.5–27.7) at 28 days after the second dose in the day 0 and 14 vaccination cohort. Meanwhile, the seroconversion rate was 97% with a GMT of 44.1 (95% CI 37.2–52.2) at 28 days after the second dose in the day 0 and 28 vaccination cohort. However, based on the phase I/II clinical trial results, this study used the emergency vaccination schedule (day 0 and 14) which may be suitable for emergency use during the COVID-19 pandemic since antibody responses may be induced within a relatively short period of time [17].

Comparing the three different batches of vaccine (batch number 20200308, 20200412, and 20200419), we observed no significant differences in the proportion of participants with seropositive and seroconversion rates based on ELISA and neutralization assay, which demonstrated good consistency between each batch of the SARS-CoV-2 vaccine. The results of this interim report show the efficacy above the value required by the WHO [31].

Currently this study is still on-going to evaluate antibody persistence and efficacy up to 6 months after the second dose of vaccine. One limitation of our study is that it only assesses the efficacy of healthy adults aged 18–59 years with a limited number of subjects. Therefore, it still requires further research to obtain vaccine efficacy, safety, and immunogenicity data in the population aged 60 years of age and over, with or without comorbidities.

5. Conclusion

Based on the interim analysis, the vaccine showed a 65.30% efficacy at preventing COVID-19 illness with a good safety and immunogenicity profile.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank all the subjects who participated in this study, the head of the Bandung District Health Office, the Dean of Faculty of Medicine Universitas Padjadjaran, the head and staff of Puskesmas Garuda, Puskesmas Dago, Puskesmas Ciumbuleuit, Puskesmas Sukapakir, Klinik Kesehatan UNPAD, for their support.

We would also like to thank our Sub-Investigators, Susi Susanah, Prayudi Santoso, Rudi Wicaksana, Yovita Hartantri, Hendarsyah S., Viramitha Kusnandi Rusmil, Reni Ghrahani, Mia Milanti Dewi, Akbar Tirtosudiro, Riyadi, Yudith Ermaya, Fina Meilyana Andriyani for their valuable contribution to the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.09.052.

References

[1] Ophinni Y, Hasibuan AS, Widhani A, Maria S, Koesnoe S, Yunihastuti E, et al. COVID-19 Vaccines: Current Status and Implication for Use in Indonesia. Acta Med Indones 2020;52:388–412.
[2] WHO. Coronavirus disease (COVID-19). Situation Report-132. 2020.
[3] Gupta SD. Coronavirus Pandemic: A Serious Threat to Humanity. J Health Manag 2020;22(1):1–2. https://doi.org/10.1177/0972063420921260.
[4] Zhu Na, Zhang D, Wang W, Li X, Yang Bo, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med 2020;382(8):727–33. https://doi.org/10.1056/NEJMoa2001017.
[5] García-Basteiro AL, Chaccour C, Guinovart C, Llupià A, Brew J, Trilla A, et al. Monitoring the COVID-19 epidemic in the context of widespread local transmission. Lancet Respir Med 2020;8(5):440–2. https://doi.org/10.1016/S2213-2600(20)30162-4.
[6] WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020 n.d. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—11-march-2020 (accessed January 23, 2021).
[7] World Health Organization. Weekly Epidemiological Update on COVID-19. World Heal Organ 2020;1:4.
[8] WHO. Situation Report-7 INDONESIA Situation Report 19 Internal for SEARO. 2020.
[9] Wenjiang Fu1 2*, Jieni Li3 and Paul Scheet 4. Covid-19 Vaccine Efficacy: Accuracy, Uncertainty and Projection of Cases. MedRxiv 2020. 10.1101/2020.12.16.20248359.
[10] Lurie N, Saville M, Hatchett R, Halton J. Developing Covid-19 Vaccines at Pandemic Speed. N Engl J Med 2020;382(21):1969–73. https://doi.org/10.1056/NEJMp2005630.
[11] WHO. WHO R&D Blueprint novel Coronavirus Outline of designs for experimental vaccines and therapeutics. vol. 205. 2020.
[12] Wang H, Zhang Y, Huang B, Deng W, Quan Y, Wang W, et al. Development of an Inactivated Vaccine Candidate, BBIBP-CorV, with Potent Protection against SARS-CoV-2. Cell 2020;182(3):713–721.e9. https://doi.org/10.1016/j.cell.2020.06.008.
[13] Vellozzi C, Burwen DR, Dobardzic A, Ball R, Walton K, Haber P. Safety of trivalent inactivated influenza vaccines in adults: Background for pandemic influenza vaccine safety monitoring. Vaccine 2009;27(15):2114–20. https://doi.org/10.1016/j.vaccine.2009.01.125.
[14] Murdin AD, Barreto L, Plotkin S. Inactivated poliovirus vaccine: Past and present experience. Vaccine 1996;14(8):735–46. https://doi.org/10.1016/0264-410X(95)00211-I.
[15] (WHO) WHO. Draft landscape and tracker of COVID-19 candidate vaccines 2021. https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines (accessed January 25, 2021).
[16] Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science (80-) 2020;369(6499):77–81. https://doi.org/10.1126/science:abc1932.
[17] Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Articles Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years. Lancet Infect Dis 2020.
[18] KEMENKES. Keputusan Menteri Kesehatan Republik Indonesia Nomor HK.01.07/MenKes/413/2020 Tentang Pedoman Pencegahan dan Pengendalian Corona Virus Disease 2019 (Covid-19). MenKes/413/2020 2020;2019:207.
[19] Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease Patients. Emerg Infect Dis 2020;26(7):1478–88. https://doi.org/10.3201/eid2607.200841.
[20] Jiang S, Hillyer C, Du L. Neutralizing Antibodies against SARS-CoV-2 and Other Human Coronaviruses. Trends Immunol 2020;41(5):355–9. https://doi.org/10.1016/j.it.2020.03.007.
[21] Grzelak L, Temmam S, Planchais C, Demeret C, Huon C, Guivel-Benhassine F, et al. SARS-CoV-2 serological analysis of COVID-19 hospitalized patients, pauci-symptomatic individuals and blood donors. MedRxiv 2020;17:25. https://doi.org/10.1101/2020.04.21.20068858.
[22] SAGE (Strategic Advisory Group of Experts). Background document on the inactivated vaccine Sinovac-CoronaVac against COVID-19. 2021.

[23] Tanriover MD, Doğanay HL, Akova M, Güner HR, Azap A, Akhan S, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. Lancet 2021;398:213–22. 10.1016/S0140-6736(21)01429-X.
[24] Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. N Engl J Med 2021;385(10):875–84. https://doi.org/10.1056/NEJMoa2107715.
[25] Sinovac Announces Phase III Results of Its COVID-19 Vaccine-SINOVAC - Supply Vaccines to Eliminate Human Diseases n.d. http://www.sinovac.com/?optionid=754&auto_id=922 (accessed March 17, 2021).
[26] CDC. Principles of Epidemiology | Lesson 3 - Section 6 n.d. https://www.cdc.gov/csels/dsepd/ss1978/lesson3/section6.html (accessed February 22, 2021).
[27] Fda, Cber. Contains Nonbinding Recommendations Development and Licensure of Vaccines to Prevent COVID-19 Guidance for Industry. 2020.
[28] Fda, Cber. Contains Nonbinding Recommendations Emergency Use Authorization for Vaccines to Prevent COVID-19 Guidance for Industry Preface Public Comment. 2020.
[29] Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet 2020;396:467–78. https://doi.org/10.1016/S0140-6736(20)31604-4.
[30] Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine _ Enhanced Reader.pdf. N Engl J Med 2020;383(27):2603–15.
[31] (WHO) WHO. CONSIDERATIONS FOR EVALUATION OF COVID19 VACCINES 2020;21:1–14. https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines (accessed January 25, 2021).

1.14. CoronaVac promove alta resposta imune humoral e celular, mostra estudo chileno

Uma pesquisa chilena publicada na revista Clinical Infectious Diseases atestou a segurança e imunogenicidade da CoronaVac em adultos saudáveis, mostrando que a vacina induz uma elevada resposta imune celular e humoral (produção de anticorpos). Divulgado em setembro de 2021, o estudo foi conduzido por pesquisadores da Pontifícia Universidade Católica do Chile.

Foram acompanhados 434 voluntários, sendo 397 com idades entre 18 e 59 anos e 37 com mais de 60 anos. Entre os participantes, 390 tomaram duas doses do imunizante e 44 receberam placebo. Não foi relatado nenhum efeito adverso grave e os principais sintomas foram dor no local da injeção e dor de cabeça.

A avaliação da resposta imune humoral foi feita em 81 voluntários. Um mês após a segunda dose da vacina, a taxa de soroconversão de anticorpos IgG específicos para o domínio de ligação ao receptor (RBD) da proteína Spike do SARS-CoV-2 foi de 84,4% para indivíduos entre 18 e 59 anos e de 70,3% para os idosos. Também foi detectado um aumento na circulação de anticorpos neutralizantes.

Os cientistas avaliaram, ainda, a resposta imune celular em 47 participantes. Foi detectada uma resposta significativa de células T, caracterizada pela secreção dos interferon-gama (IFN-γ) – citocinas que ativam os macrófagos, importantes células de defesa do organismo.

"Os resultados indicam que a CoronaVac é segura e induz respostas humoral e celular robustas, produzindo anticorpos específicos para RBD com capacidade de neutralização e ativando as células T", conclui o estudo.

Publicado em: 19/09/2021

Clinical Infectious Diseases

MAJOR ARTICLE

Safety and Immunogenicity of an Inactivated Severe Acute Respiratory Syndrome Coronavirus 2 Vaccine in a Subgroup of Healthy Adults in Chile

Susan M. Bueno,[1,2,a] Katia Abarca,[1,3] Pablo A. González,[1,2] Nicolás M. S. Gálvez,[1,2] Jorge A. Soto,[1,2] Luisa F. Duarte,[1,2] Bárbara M. Schultz,[1,2] Gaspar A. Pacheco,[1,2] Liliana A. González,[1,2] Yaneisi Vázquez,[1,2] Mariana Ríos,[1,2] Felipe Melo-González,[1,2] Daniela Rivera-Pérez,[1,2] Carolina Iturriaga,[3] Marcela Urzúa,[3] Angélica Domínguez,[4] Catalina A. Andrade,[1,2] Roslye V. Berríos-Rojas,[1,2] Gisela Canedo-Marroquín,[1,2] Camila Covián,[1,2] Daniela Moreno-Tapia,[1,2] Farides Saavedra,[1,2] Omar P. Vallejos,[1,2] Paulina Donato,[5] Pilar Espinoza,[6,7] Daniela Fuentes,[8,10] Marcela González,[9,10] Paula Guzmán,[11] Paula Muñoz Venturelli,[12,13] Carlos M. Pérez,[6,7] Marcela Potin,[14] Álvaro Rojas,[15] Rodrigo A. Fasce,[16] Jorge Fernández,[16] Judith Mora,[16] Eugenio Ramírez,[16] Aracelly Gaete-Argel,[17] Aarón Oyarzún-Arrau,[17] Fernando Valiente-Echeverría,[17] Ricardo Soto-Rifo,[17] Daniela Weiskopf,[18] Alessandro Sette,[18,19] Gang Zeng,[20] Weining Meng,[20] José V. González-Aramundiz,[21] and Alexis M. Kalergis[1,2,21,22,a]; on behalf of CoronaVac03CL Study Group

[1]Millennium Institute on Immunology and Immunotherapy, Santiago, Chile; [2]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile; [3]Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, División de Pediatría, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile; [4]Departamento de Salud Pública, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile; [5]Complejo Asistencial Dr. Sótero del Rio, Santiago, Chile; [6]Hospital Clínico Félix Bulnes, Santiago, Chile; [7]Facultad de Medicina y Ciencia y Facultad de Ciencias para el Cuidado de la Salud. Universidad San Sebastián, Santiago, Chile; [8]Hospital Carlos Van Buren, V Región, Chile; [9]Hospital Dr. Gustavo Fricke, V Región, Chile; [10]Departamento de Pediatría, Universidad de Valparaíso, Valparaiso, Chile; [11]Clínica Los Andes, Universidad de Los Andes, Santiago, Chile; [12]Centro de Estudios Clínicos, Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina Clínica Alemana Universidad del Desarrollo, Santiago, Chile; [13]The George Institute for Global Health, Faculty of Medicine, University of New South Wales, Sydney, Australia; [14]Clínica San Carlos de Apoquindo, Red de Salud UC Christus, Santiago, Chile; [15]Departamento de Enfermedades Infecciosas del Adulto, División de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile; [16]Departamento de Laboratorio Biomédico, Instituto de Salud Pública de Chile, Santiago, Chile; [17]Laboratory of Molecular and Cellular Virology, Virology Program, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Santiago, Chile; [18]Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology, La Jolla, California, USA; [19]Department of Medicine, Division of Infectious Diseases and Global Public Health, University of California, San Diego (UCSD), La Jolla, CA 92037, USA; [20]Sinovac Biotech, Beijing, China; [21]Departamento de Farmacia, Facultad de Química y de Farmacia. Pontificia Universidad Católica de Chile, Santiago, Chile; and [22]Departamento de Endocrinología, Facultad de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

Background. The development of effective vaccines against coronavirus disease 2019 is a global priority. CoronaVac is an inactivated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine with promising safety and immunogenicity profiles. This article reports safety and immunogenicity results obtained for healthy Chilean adults aged ≥18 years in a phase 3 clinical trial.

Methods. Volunteers randomly received 2 doses of CoronaVac or placebo, separated by 2 weeks. A total of 434 volunteers were enrolled, 397 aged 18–59 years and 37 aged ≥60 years. Solicited and unsolicited adverse reactions were registered from all volunteers. Blood samples were obtained from a subset of volunteers and analyzed for humoral and cellular measures of immunogenicity.

Results. The primary adverse reaction in the 434 volunteers was pain at the injection site, with a higher incidence in the vaccine than in the placebo arm. Adverse reactions observed were mostly mild and local. No severe adverse events were reported. The humoral evaluation was performed on 81 volunteers. Seroconversion rates for specific anti-S1-receptor binding domain (RBD) immunoglobulin G (IgG) were 82.22% and 84.44% in the 18–59 year age group and 62.69% and 70.37% in the ≥60 year age group, 2 and 4 weeks after the second dose, respectively. A significant increase in circulating neutralizing antibodies was detected 2 and 4 weeks after the second dose. The cellular evaluation was performed on 47 volunteers. We detected a significant induction of T-cell responses characterized by the secretion of interferon-γ (IFN-γ) upon stimulation with Mega Pools of peptides from SARS-CoV-2.

Conclusions. Immunization with CoronaVac in a 0–14 schedule in Chilean adults aged ≥18 years is safe, induces anti-S1-RBD IgG with neutralizing capacity, activates T cells, and promotes the secretion of IFN-γ upon stimulation with SARS-CoV-2 antigens.

Keywords. CoronaVac; phase 3 clinical trial; SARS-CoV-2; COVID-19; vaccines.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the emerging pathogen responsible for coronavirus disease 2019 (COVID-19) [1–3]. This virus was first described in December 2019 in Wuhan, China, and it is the source of an ongoing pandemic, which by September 2021 has resulted in almost 221 million infection cases and more than 4.5 million deaths worldwide [4]. International efforts are focused on generating vaccines to counteract COVID-19. Epidemiological studies show that individuals aged ≥60 years and those with chronic conditions are more susceptible to severe disease, frequently resulting in death [5, 6]. More than 294 vaccines are under development, with 37 undergoing phase 3 or 4 clinical trials and 10 approved for emergency use [7]. Although many different vaccine platforms are being

Received 26 April 2021; editorial decision 11 September 2021; published online 19 September 2021.

[a]S. M. B. and A. M. K. contributed equally to this work.

Correspondence: A. M. Kalergis, Pontificia Universidad Católica de Chile, Av. Libertador Bernardo O'Higgins Nº 340, Santiago 8331010, Santiago, Chile (akalergis@bio.puc.cl).

Clinical Infectious Diseases® 2021;XX(XX):1–13

https://doi.org/10.1093/cid/ciab823

used and explored, most of them rely on a single viral component, the full-length Spike (S) protein or the receptor binding domain (RBD) of the S protein [7, 8]. Whole virus inactivated platforms are a mature technology widely used against different viruses, and they can be easily stored and shipped at 4ºC for several years, which is a significant advantage for developing countries [9, 10]. Whole inactivated vaccines carry a wider diversity of antigens that are more prone to be conserved than the S protein in circulating variants, as is the case for the nucleocapsid (N) protein that has shown to promote protective T-cell immunity against related SARS-CoV viruses. Thus, including the N, envelope (E), and matrix (M) proteins of SARS-CoV-2 as additional antigenic targets could boost protection for whole inactivated vaccines [11].

CoronaVac is a whole inactivated SARS-CoV-2 vaccine developed by Sinovac Life Sciences Co., Ltd. (Beijing, China) [12]. Phase 1/2 clinical trials carried out in China evaluated 2 vaccination schedules with 2 doses separated by 14 days (0–14) or 28 days (0–28) [13, 14]. Both trials showed that this vaccine induces neutralizing antibodies 14 days after the second dose, suggesting that this vaccine is safe and likely induces a protective immune response against SARS-CoV-2 [13, 14]. Currently, 4 phase 3 clinical trials are evaluating the efficacy of CoronaVac and are being carried out in Brazil, Turkey, Indonesia, and Chile. Here, we report an interim analysis of safety and immunogenicity parameters upon immunization of a group of healthy Chilean adults with CoronaVac or placebo aged 18–59 years and ≥60 years in a 0–14 day vaccination schedule. The safety was evaluated in the total 434 volunteers recruited, and a subgroup was included in immunogenicity analysis. Given that this vaccine carries multiple SARS-CoV-2 antigens, the characterization of the humoral and cellular immune response was extended to components of the viral proteome beyond the S protein. Taken together, this is the first report characterizing the cellular and humoral immune responses elicited by CoronaVac in a population other than the Chinese against several viral antigens. Our results indicate that CoronaVac is safe and immunogenic in healthy Chilean adults.

MATERIALS AND METHODS

Study Design, Randomization, and Volunteers

This clinical trial (clinicaltrials.gov NCT04651790) was conducted in Chile at 8 different sites. The study protocol was performed according to the current Tripartite Guidelines for Good Clinical Practices, the Declaration of Helsinki [15], and local regulations. The trial protocol was reviewed and approved by the Institutional Scientific Ethical Committee of Health Sciences, Pontificia Universidad Católica de Chile (#200708006). Trial execution was approved by the Chilean Public Health Institute (#24204/20). Written informed consent was obtained from each volunteer before enrollment. The study included healthy Chilean adults aged ≥18 years. Volunteers were inoculated with either 2 doses of CoronaVac or placebo separated by 2 weeks.

A complete list of inclusion/exclusion criteria is provided in the annexed study protocol. Volunteers were randomly assigned to immunization with CoronaVac or injection with placebo in a 1:1 ratio. A subgroup of volunteers was assigned to the immunogenicity arm and randomly received CoronaVac or placebo (3:1 ratio). Randomization was done using a sealed enveloped system integrated into the electronic case report forms in the OpenClinica platform. To collect adverse events (AEs), volunteers were instructed and trained to log in information on the platform until 28 days after the second dose at the same hour each day. Local and systemic symptoms were requested for 7 days after each dose or until they ceased. Other AEs, drugs used, severe adverse events (SAEs), events of special interest, and symptoms of SARS-CoV-2 were also requested until the end of the study. Daily reminders were sent via email and SMS until 28 days after the second dose and then weekly until the end of the study. Table 1 summarizes the characteristics of the volunteers, and Figure 1 shows the study profile.

Table 1. Characteristics of the Volunteers at Baseline

Characteristic	18–59 y (n = 397)	≥ 60 y (n = 37)	Total (n = 434)	*P* Value
Age, mean ± standard deviation	38.2 ± 9.7	64.0 ± 4.3	40.4 ± 11.8	
Inoculation				.482
Vaccine, n (%)	245 (61.7)	25 (67.6)	270 (62.2)	
Placebo, n (%)	152 (38.3)	12 (32.4)	164 (37.8)	
Sex				.039
Female, n (%)	251 (63.2)	17 (45.9)	268 (61.8)	
Male, n (%)	146 (36.8)	20 (54.1)	166 (38.2)	
Ethnicity				.152
White, n (%)	370 (93.2)	37 (100.0)	407 (93.8)	
Other, n (%)	27 (6.8)	0 (0.0)	27 (6.2)	

P values are for comparison between total numbers in each characteristic.

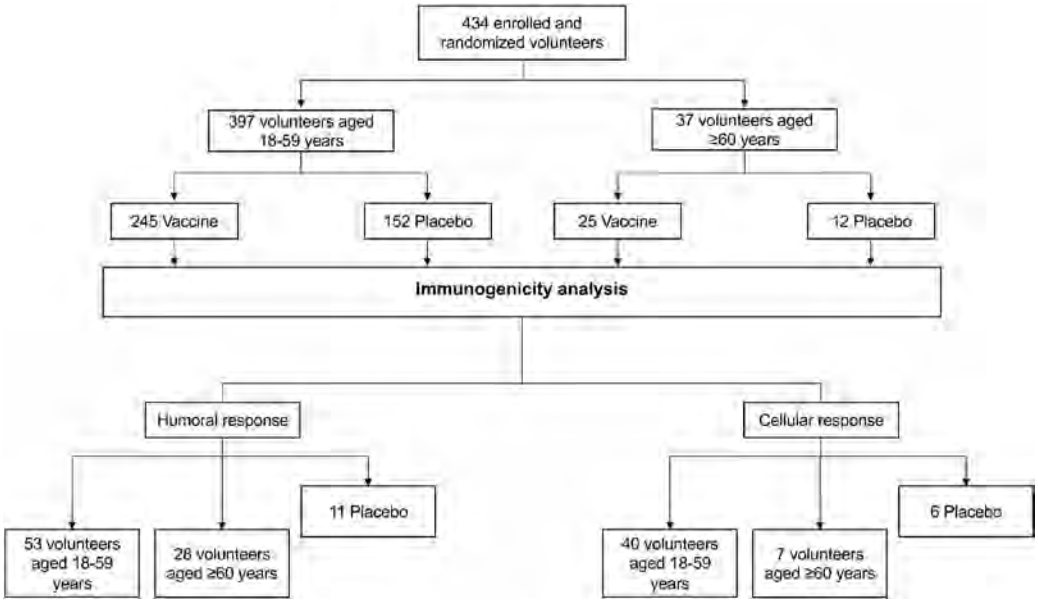


Figure 1. Study profile. Recruitment of volunteers for the phase 3 clinical trial as of February 10, 2021.

Procedures

CoronaVac consists of 3 μg of β-propiolactone inactivated SARS-CoV-2 (strain CZ02) with aluminum hydroxide as an adjuvant in 0.5 mL [12]. A study nurse administered unblinded ready-to-use syringes with CoronaVac or placebo (visually indistinguishable among them) intramuscularly in the deltoid area. To avoid any influence on the volunteers, the interaction with the nurse was restricted only to immunization. Then, safety evaluations were performed by the blinded clinical team. Blood samples were obtained at different time points for the immunogenicity arm and used to isolate sera and peripheral blood mononuclear cells (PBMCs). Further details can be found in the supplementary information.

To assess the presence of anti-SARS-CoV-2 antibodies, blood samples obtained before the first and second dose and 2 and 4 weeks after the second dose were analyzed. The quantitative measurement of human immunoglobulin G (IgG) antibodies against the RBD of the S1 protein (S1-RBD) and the N protein of SARS-CoV-2 was performed using the RayBio COVID-19 (SARS-CoV-2) Human Antibody Detection Kit (catalog #IEQ-CoVS1RBD-IgG and #IEQ-CovN-IgG). Arbitrary units obtained for these analyses were converted into World Health Organization (WHO) international units through a standard curve (National Institute for Biological Standards and Control code 20/268). The neutralizing capacities of circulating antibodies were evaluated by 3 different techniques: surrogate virus neutralization test (sVNT) (Genscript catalog #L00847-A), conventional virus neutralization test (cVNT), and pseudotyped virus neutralization test (pVNT) [16]. Further details on the methodology associated with these techniques can be found in the supplementary information.

To assess the cellular immune response, enzyme-linked immunospot (ELISPOT) and flow cytometry assays were performed using isolated PBMCs. ELISPOT assays were performed to evaluate changes in the numbers of interferon-γ (IFN-γ) secreting cells. Flow cytometry assays were performed to characterize T cells and the expression of activation-induced markers (AIMs) on these cells. The stimulus included in these assays considered the use of Mega Pools (MPs) of peptides derived from SARS-CoV-2 proteins [17]. Corresponding controls were held. Further details on the ELISPOT assays, antibodies used for flow cytometry, and the respective protocols can be found in the supplementary information.

Outcomes

The primary aim was to evaluate the frequency of solicited and unsolicited AEs occurring 7 days after each dose by age group (aged 18–59 and ≥60 years). Grading for solicited and unsolicited AEs can be found in detail in Tables S1–S4. Secondary immunogenicity endpoints considered assessing the presence of anti-SARS-CoV-2 antibodies and the cellular immune response elicited by the vaccine in a subgroup of volunteers. A complete list of outcomes can be found in the study protocol.

Statistical Analysis

Information regarding the determination of sample size, AE analysis test, and immunogenicity analysis test can be found in the supplementary information.

	First Dose			Second Dose			Both Doses		
	(n = 434)			(n = 319)			(n = 319)		
Adverse Reaction	Placebo (n = 164)	Vaccine (n = 270)	*P* Value	Placebo (n = 80)[a]	Vaccine (n = 239)	*P* Value	Placebo (n = 80)	Vaccine (n = 239)	*P* Value
Local reactions									
Pain, n (%)[b]	39 (23.8)	117 (43.3)	<.001	16 (20.0)	73 (30.5)	.069	32 (40.0)	133 (55.6)	.015
<60 y	37 (24.3)	113 (46.1)	<.001	15 (20.8)	68 (31.8)	.077	30 (41.7)	125 (58.4)	.014
≥60 y	2 (16.7)	4 (16.0)	.999	1 (12.5)	5 (20.0)	.999	2 (25.0)	8 (32.0)	.999
Induration (%)	1 (0.6)	8 (3.0)	.163	0 (0.0)	15 (6.3)	.015	0 (0.0)	21 (8.8)	.006
<60 y	1 (0.7)	7 (2.9)	.161	0 (0.0)	13 (6.1)	.043	0 (0.0)	18 (8.4)	.009
≥60 y	0 (0.0)	1 (4.0)	.999	0 (0.0)	2 (8.0)	.999	0 (0.0)	3 (12.0)	.56
Pruritus (%)	4 (2.4)	15 (5.6)	.124	2 (2.5)	6 (2.5)	.099	3 (3.8)	17 (7.1)	.283
<60 y	4 (2.6)	15 (6.1)	.113	2 (2.8)	6 (2.8)	.099	3 (4.2)	17 (7.9)	.277
≥60 y	0 (0.0)	0 (0.0)	…	0 (0.0)	0 (0.0)	…	0 (0.0)	0 (0.0)	…
Erythema (%)	3 (1.8)	10 (3.7)	.386	2 (2.5)	3 (1.3)	.602	2 (2.5)	10 (4.2)	.737
<60 y	3 (2.0)	10 (4.1)	.385	1 (1.4)	3 (1.4)	.999	1 (1.4)	10 (4.7)	.301
≥60 y	0 (0.0)	0 (0.0)	…	1 (12.5)	0 (0.0)	.242	1 (12.5)	0 (0,0.0)	.242
Swelling (%)	3 (1.8)	5 (1.9)	.999	1 (1.3)	5 (2.1)	.999	1 (1.3)	9 (3.8)	.461
<60 y	3 (2.0)	5 (2.0)	.999	1 (1.4)	4 (1.9)	.999	1 (1.4)	8 (3.7)	.458
≥60 y	0 (0.0)	0 (0.0)	…	0 (0.0)	1 (4.0)	.999	0 (0.0)	1 (4.0)	.999
Systemic reactions									
Headache (%)	50 (30.5)	107 (39.6)	.055	15 (18.8)	46 (19.2)	.922	39 (48.8)	116 (48.5)	.974
<60 y	49 (32.2)	102 (41.6)	.061	12 (16.7)	42 (19.6)	.579	36 (50.0)	109 (50.9)	.891
≥60 y	1 (8.3)	5 (20.0)	.641	3 (37.5)	4 (16.0)	.32	3 (37.5)	7 (28.0)	.673
Fatigue (%)	32 (19.5)	58 (21.5)	.624	10 (12.5)	25 (10.5)	.613	22 (27.5)	64 (26.8)	.9
<60 y	31 (20.4)	55 (22.4)	.629	8 (11.1)	23 (10.7)	.932	20 (27.8)	60 (28.0)	.966
≥60 y	1 (8.3)	3 (12.0)	.999	2 (25.0)	2 (8.0)	.241	2 (25.0)	4 (16.0)	.616
Myalgia (%)	23 (14.0)	48 (17.8)	.305	9 (11.3)	19 (7.9)	.367	19 (23.8)	54 (22.6)	.831
<60 y	22 (14.5)	46 (18.8)	.269	8 (11.1)	16 (7.5)	.336	18 (25.0)	50 (23.4)	.778
≥60 y	1 (8.3)	2 (8.0)	.999	1 (12.5)	3 (12.0)	.999	1 (12.5)	4 (16.0)	.999
Diarrhea (%)	18 (11.0)	36 (13.3)	.471	5 (6.3)	18 (7.5)	.701	15 (18.8)	44 (18.4)	.946
<60 y	17 (11.2)	36 (14.7)	.318	4 (5.6)	16 (7.5)	.58	14 (19.4)	42 (19.6)	.973
≥60 y	1 (8.3)	0 (0.0)	.324	1 (12.5)	2 (8.0)	.999	1 (12.5)	2 (8.0)	.999
Nausea (%)	18 (11.0)	25 (9.3)	.562	3 (3.8)	9 (3.8)	.999	11 (13.8)	27 (11.3)	.558
<60 y	18 (11.8)	22 (9.0)	.357	2 (2.8)	9 (4.2)	.736	10 (13.9)	24 (11.2)	.544
≥60 y	0 (0.0)	3 (12.0)	.537	1 (12.5)	0 (0.0)	.242	1 (12.5)	3 (12.0)	.999
Arthralgia (%)	10 (6.1)	14 (5.2)	.687	2 (2.5)	7 (2.9)	.999	7 (8.8)	18 (7.5)	.726
<60 y	10 (6.6)	13 (5.3)	.596	2 (2.8)	6 (2.8)	.999	7 (9.7)	16 (7.5)	.544
≥60 y	0 (0.0)	1 (4.0)	.999	0 (0.0)	1 (4.0)	.999	0 (0.0)	2 (8.0)	.999
Anorexia (%)	10 (6.1)	18 (6.7)	.815	3 (3.8)	3 (1.3)	.169	6 (7.5)	16 (6.7)	.806

Table 2. Continued

	First Dose (n = 434)			Second Dose (n = 319)			Both Doses (n = 319)		
Adverse Reaction	Placebo (n = 164)	Vaccine (n = 270)	*P* Value	Placebo (n = 80)[a]	Vaccine (n = 239)	*P* Value	Placebo (n = 80)	Vaccine (n = 239)	*P* Value
<60 y	10 (6.6)	16 (6.5)	.985	2 (2.8)	3 (1.4)	.603	5 (6.9)	14 (6.5)	.999
≥60 y	0 (0.0)	2 (8.0)	.999	1 (12.5)	0 (0.0)	.242	1 (12.5)	2 (8.0)	.999
Pruritus (%)	2 (1.2)	10 (3.7)	.225	0 (0.0)	4 (1.7)	.575	1 (1.3)	14 (5.9)	.127
<60 y	2 (1.3)	9 (3.7)	.217	0 (0.0)	4 (1.9)	.575	1 (1.4)	13 (6.1)	.202
≥60 y	0 (0.0)	1 (4.0)	.999	0 (0.0)	0 (0.0)	…	0 (0.0)	1 (4.0)	.999
Exanthema (%)	1 (0.6)	7 (2.6)	.268	0 (0.0)	1 (0.4)	.999	1 (1.3)	8 (3.3)	.459
<60 y	1 (0.7)	7 (2.9)	.161	0 (0.0)	1 (0.5)	.999	1 (1.4)	8 (3.7)	.458
≥60 y	0 (0.0)	0 (0.0)	…	0 (0.0)	0 (0.0)	…	0 (0.0)	0 (0.0)	…
Allergy (%)	1 (0.6)	6 (2.2)	.262	0 (0.0)	3 (1.3)	.575	0 (0.0)	8 (3.3)	.209
<60 y	0 (0.0)	5 (2.0)	.161	0 (0.0)	2 (0.9)	.999	0 (0.0)	4 (1.9)	.575
≥60 y	1 (8.3)	1 (4.0)	.999	0 (0.0)	1 (4.0)	.999	0 (0.0)	1 (4.0)	.999
Vomiting (%)	3 (1.8)	1 (0.4)	.154	0 (0.0)	4 (1.7)	.575	0 (0.0)	4 (1.7)	.575
<60 y	3 (2.0)	1 (0.4)	.159	0 (0.0)	4 (1.9)	.575	0 (0.0)	4 (1.9)	.575
≥60 y	0 (0.0)	0 (0.0)	…	0 (0.0)	0 (0.0)	…	0 (0.0)	0 (0.0)	…
Fever (>37.8°C) (%)	1 (0.6)	1 (0.4)	.999	0 (0.0)	0 (0.0)	…	1 (1.3)	1 (0.4)	.439
<60 y	1 (0.7)	1 (0.4)	.999	0 (0.0)	0 (0.0)	…	1 (1.4)	1 (0.5)	.441
≥60 y	0 (0.0)	0 (0.0)	…	0 (0.0)	0 (0.0)	…	0 (0.0)	0 (0.0)	…

Data in the table were reported within 7 days after any of the 2 doses. Sample sizes: placebo < 60 first dose: n = 152; placebo < 60 second dose: n = 72; vaccine < 60 first dose: n = 245; vaccine < 60 second dose: n = 214; placebo ≥ 60 first dose: n = 12; placebo ≥ 60 second dose: n = 8; vaccine ≥ 60 first dose: n = 25; vaccine ≥ 60 years second dose: n = 25.

[a]As of February 24, 2021, only 80 volunteers from the placebo arm had received their second dose.

[b]Percentages were calculated from the total number of volunteers in each group.

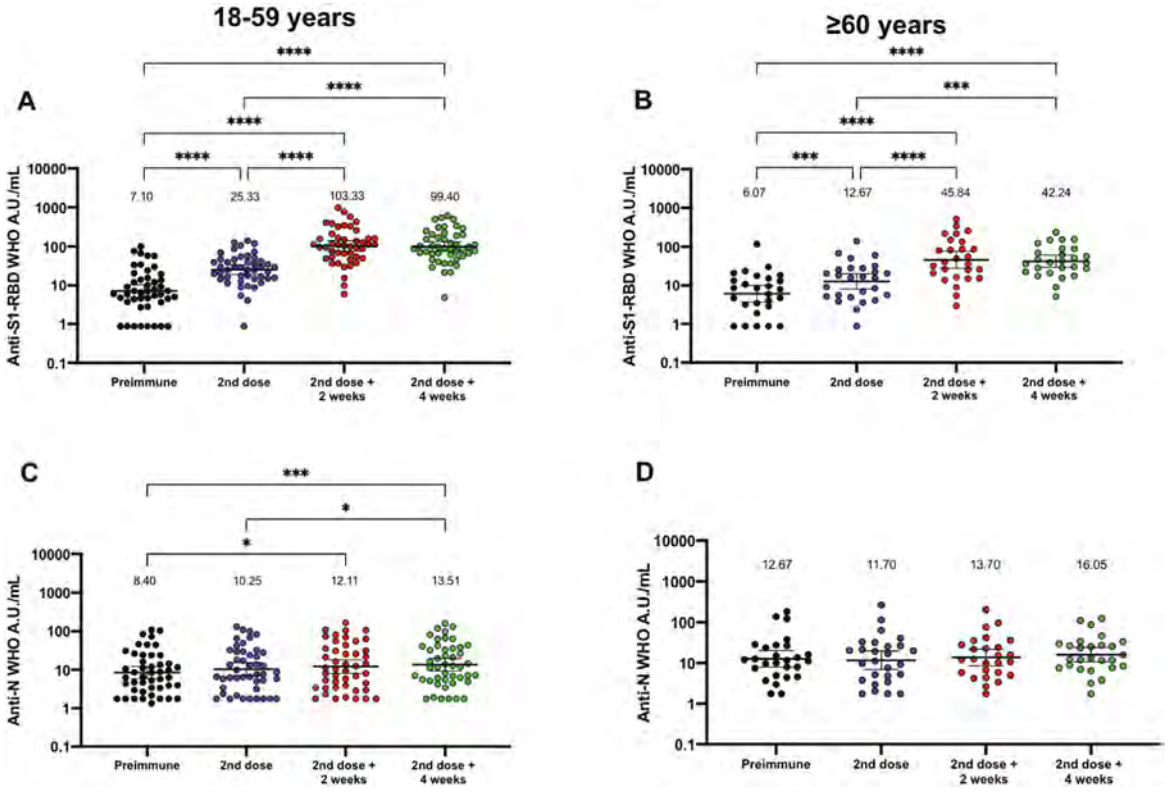


Figure 2. Immunization with CoronaVac induces specific IgG against SARS-CoV-2 antigens in participants aged 18–59 years and ≥ 60 years after 2 immunizations in a 0–14 schedule. Titers of IgG antibodies after 2 doses of CoronaVac were evaluated for immunized participants (excluding seropositive participants at recruitment and placebo participants) before the first and second dose, and 2 (second dose + 2 weeks) and 4 weeks (second dose + 4 weeks) after the second dose for adults aged (*A, C*) 18–59 years and (*B, D*) ≥60 years. Specific IgG against the S1-RBD (upper panel) and the N protein (lower panel) of SARS-CoV-2 were measured. Data are expressed as the $\log_{10}$ of international WHO arbitrary units versus time after each dose. Error bars indicate the 95% CI of the geometric mean units (GMUs). The spots represent the individual values of antibody units for each volunteer, with the numbers above each time showing the GMU estimates. The graph illustrates the results obtained for 45 participants in the 18–59 years group and 27 participants in the ≥60 years group. One-way ANOVAs with repeated measures and post hoc Tukey tests were performed to evaluate statistical differences among the groups; **P* < .05, ***P* < .005, ****P* < .0005, *****P* < .0001. Abbreviations: ANOVA, analysis of variance; CI, confidence interval; IgG, immunoglobulin G; N, nucleocapsid; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

RESULTS

Safety Assessment

Volunteers on this study were recruited between November 27, 2020, and February 10, 2021 (Figure 1). On February 24, 2021, the last volunteer included in this analysis was inoculated with the second dose. As of February 24, 2021, only 80 volunteers from the placebo arm had received their second dose. Circulating SARS-CoV-2 strains detected during this time mainly were wild-type strains (original L strain) and the B.1.1.7 strain. Remarkably, the P1 or Gamma variant was detected for the first time in Chile by the end of January 2021 [18]. A total of 434 volunteers were enrolled in this study; 390 volunteers received 2 doses of CoronaVac and 44 received a placebo. The vaccination schedule for both groups was 0–14. A list of local and systemic solicited AEs reported is shown in Table 2. The most reported solicited local AEs was pain at the injection site (mostly grade 1), with an incidence of 55.6% in the vaccine arm compared with 40.0% in the placebo arm. Headaches (grade 1 or 2) were the most common solicited systemic AEs with a frequency of 48.5% in the vaccine arm and 48.8% in the placebo arm. No SAEs or events of special interest were reported. Significant differences were observed between age groups regarding the frequency of local and systemic AEs (Table S4). A total of 55 unsolicited AEs were reported. During the study period, 3 COVID-19 cases occurred in the vaccinated group (breakthrough cases). One of them had a clinical progression score of 1 (asymptomatic), and the other 2 had a score of 2 (symptomatic) [19].

Immunization With CoronaVac Induces the Secretion of anti-S1-RBD IgG, anti-N IgG, and Circulating Neutralizing Antibodies in Chilean Adults

Evaluation of IgG-specific against S1-RBD and the N protein of SARS-CoV-2 was performed independently through enzyme-linked immunosorbent assays (Figure 2). This humoral

Table 3. Seroconversion Rates and Geometric Median Units (GMU) of Circulating Antibodies Against SARS-CoV-2 Proteins

Antibodies Detected	Group	Indicators	Second Dose	Second Dose + 2 wk	Second Dose + 4 wk
Anti-S1-RBD IgG (WHO A.U./mL)	Total vaccine	Seroconversion n/N	23/72	54/72	57/72
		(%)	(31.94)	(75.00)	(79.17)
		GMU	19.60	76.50	72.43
		(95% CI)	(15.24–25.22)	(57.67–101.5)	(56.96–92.11)
	18–59 years	Seroconversion n/N	18/45	37/45	38/45
		(%)	(40.00)	(82.22)	(84.44)
		GMU	25.33	103.33	99.40
		(95% CI)	(19.07–33.64)	(75.31–141.8)	(74.53–132.6)
	≥ 60 years	Seroconversion n/N	5/27	17/27	19/27
		(%)	(18.52)	(62.96)	(70.37)
		GMU	12.67	45.84	42.24
		(95% CI)	(08.03–19.99)	(27.51–76.36)	(29.44–60.61)
	Placebo	Seroconversion n/N	0/12	0/9	0/0
		(%)	(0)	(0)	N/D
		GMU	10.43	6.19	N/D
		(95% CI)	(04.33–25.10)	(01.85–20.76)	N/D
Anti-N IgG (WHO A.U./mL)	Total vaccine	Seroconversion n/N	2/72	5/72	7/72
		(%)	(2.78)	(6.94)	(9.72)
		GMU	10.77	12.66	14.4
		(95% CI)	(07.95–14.57)	(09.36–17.12)	(10.89–19.04)
	18–59 years	Seroconversion n/N	2/45	5/45	6/45
		(%)	(4.44)	(11.11)	(13.33)
		GMU	10.25	12.11	13.51
		(95% CI)	(06.97–15.08)	(08.07–18.16)	(09.21–19.81)
	≥ 60 years	Seroconversion n/N	0/27	0/27	1/27
		(%)	(0)	(0)	(3.70)
		GMU	11.70	13.70	16.05
		(95% CI)	(06.96–19.67)	(08.60–21.82)	(10.65–24.18)
	Placebo	Seroconversion n/N	1/12	0/10	0/0
		(%)	(8.3)	(0)	(-)
		GMU	11.06	9.61	N/D
		(95% CI)	(04.03–30.35)	(02.90–31.90)	(-)

Timepoints refer to the number of days after the first dose of vaccine or placebo in the schedule.
Abbreviations: A.U., arbitrary unit; CI, confidence interval; GMU, geometric median unit; IgG, immunoglobulin G; N, nucleoprotein; N/D, not determined; RBD, receptor binding domain; S, Spike protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

evaluation was performed on serum samples from 81 volunteers, 53 of whom were aged 18–59 years, and 28 of whom were aged ≥60 years. The data are shown in international WHO arbitrary units. Increased levels of anti-S1-RBD circulating antibodies were detected at all times evaluated after the first dose for both age groups (Figure 2A and 2B). These changes were also detected in fold change analyses normalized to preimmune samples (Figure S1*A* and S1*B*). These results suggest that immunization with CoronaVac induces a significant production of S1-RBD specific IgG after vaccination with a 0–14 schedule. A modest increase in IgG specific against the N protein was detected (Figure 2C and 2D), with fold change analyses showing similar results to those for the international WHO arbitrary units (Figure S1*C* and S1*D*). We confirmed that doses of CoronaVac contain significant amounts of the N protein (Figure S2). Seroconversion rates for S1-RBD and N protein specific IgG can be found in Table 3. Results obtained for seropositive volunteers at enrollment (not included in this analysis) and breakthrough cases are shown in Table S5.

To evaluate the neutralizing capacities of circulating antibodies, sVNTs (Figure 3A and 3B), pVNTs (Figure 3C and 3D), and cVNTs for the D614G variant (Figure 3E and 3F) were performed. This additional humoral evaluation was performed on serum samples from the same 81 volunteers, 53 of whom were aged 18–59 years, and 28 of whom were aged ≥60 years. Both sVNTs and cVNTs showed a significant increase in the neutralizing (or surrogate neutralizing) capacities of circulating antibodies against SARS-CoV-2 2 and 4 weeks after the second dose. This could also be detected in fold change analyses (Figures S3 and S4). The geometric mean titers and seropositivity rates for the sVNT, pVNT, and cVNT can be found in Table 4. These results suggest that immunization with CoronaVac in a 0–14 schedule promotes anti-S1-RBD IgG with neutralizing capacities in both age groups.

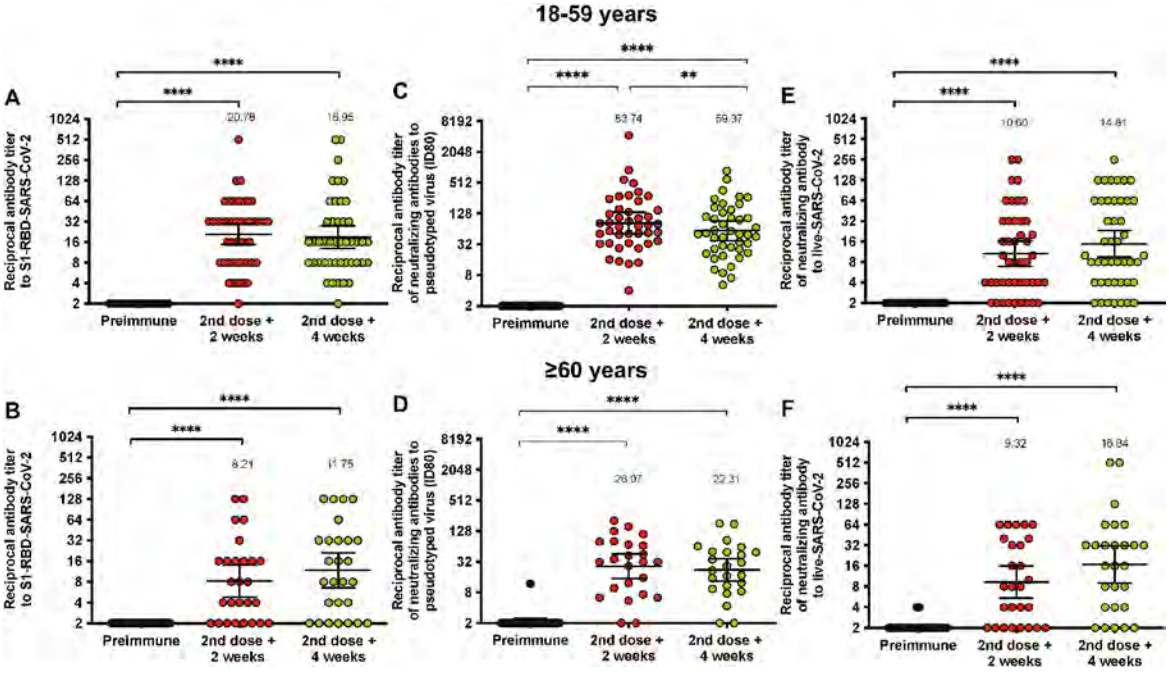


Figure 3. Immunization with CoronaVac induces neutralizing antibodies against SARS-CoV-2 in participants aged 18–59 years and ≥60 years after 2 immunizations in a 0–14 schedule. (*A-B*) Neutralizing antibody titers were evaluated with a surrogate virus neutralization assay, which quantifies the interaction between the S1-RBD and hACE2 precoated on enzyme-linked immunosorbent assay plates. Results were obtained from (*A*) 45 participants aged 18–59 years and (*B*) 27 ≥ 60 years before the first and second dose, and 2 (second dose + 2 weeks) and 4 weeks (second dose + 4 weeks) after the second dose. (*C-D*) Titers of neutralizing antibodies were evaluated with a pseudotyped viral system. Data are represented as the reciprocal dilution of sera that prevented infection by 80% (ID80) after the first dose. Numbers above the bars show the geometric mean titer (GMT), and the error bars indicate the 95% CI. Results were obtained from 45 participants (*C*) aged 18–59 years and (*D*) 24 ≥ 60 years before the first and second dose, and 2 (second dose + 2 weeks) and 4 weeks (second dose + 4 weeks) after the second dose. (*E-F*) Titers of neutralizing antibodies evaluated with a conventional neutralization assay using an ancestral D614G variant strain of SARS-CoV-2. Data are represented as the reciprocal dilution of sera that prevented infection after the first dose. Numbers above the bars show the GMT, and the error bars indicate the 95% CI. Results were obtained from 45 participants aged (*E*) 18–59 year and (*F*) 27 ≥ 60 years before the first and second dose, and 2 (second dose + 2 weeks) and 4 weeks (second dose + 4 weeks) after the second dose. Data are represented as the reciprocal antibody titer versus time after each dose. Numbers above the bars show the GMT, and the error bars indicate the 95% CI. Data were analyzed by a Wilcoxon test to evaluate statistical differences among the groups; **P* < .05, ***P* < .005, ****P* < .0005, *****P* < .0001. Abbreviations: CI, confidence interval; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Immunization With CoronaVac Induces IFN-γ-Producing T cells Specific for SARS-CoV-2 Antigens in Chilean Adults

To evaluate the cellular immune response elicited upon vaccination with CoronaVac, the specific T-cell responses induced upon stimulation of PBMCs with MPs of 15-mer peptides derived from the S protein of SARS-CoV-2 (MP-S) and the remaining proteins of this virus (MP-R) were evaluated by ELISPOT in a total of 47 volunteers. Representative images of spot forming cells (SFCs) are shown (Figure 4A). We observed an increase in the number of SFCs for IFN-γ 2 and 4 weeks after the second dose (Figure 4D). Individual data from these MP also resulted in partial increases in SFC numbers (Figure 4B and 4C). Similar trends were observed with fold change analyses (Figure S5). The specific T-cell responses against MPs of 9- to 11-mer peptides from the whole proteome of SARS-CoV-2 (MP-CD8A and MP-CD8B) were also evaluated in 27 volunteers. Stimulation with these MPs resulted in a modest nonstatistically significant increase in SFCs for IFN-γ (Figure 4E and 4G). There was a subtle fold increase of SFCs for IFN-γ in volunteers stimulated with these 9- to 11-mer MPs (Figure S5). No changes were detected for the placebo group (Figure S6). These results suggest that immunization with CoronaVac induces a T-cell response polarized toward a Th1 immune profile, as the secretion of interleukin-4 by T cells was mainly undetected (Figure S7). As a positive control, PBMCs from volunteers were stimulated with an MP of peptides derived from cytomegalovirus (Figure S8).

The expression of AIMs upon stimulation of PBMCs with these MPs was evaluated by flow cytometry. Because MP-S and MP-R were initially determined in silico to stimulate $CD4^+$ T cells optimally, the expression of AIMs was assessed on these cells for 43 volunteers. The gating strategy is shown in Figure 5A, and stimulation with MP-S and consolidated data from both MP-S + R resulted in increased expression of AIMs

Table 4. Seropositivity Rates and GMTs of Circulating Neutralizing Antibodies Against SARS-CoV-2 Proteins

Antibodies Detected	Group	Indicators	Second Dose + 2 wk	Second Dose + 4 wk
Surrogate virus neutralization	Total vaccine	Seropositivity n/N	63/72	59/72
		(%)	(87.5)	(81.94)
		GMT	14.23	15.54
		(95% CI)	(10.54–19.21)	(11.23–21.51)
	18–59 y	Seropositivity n/N	44/45	39/45
		(%)	(97.78)	(86.67)
		GMT	20.78	18.95
		(95% CI)	(14.81–29.18)	(12.87–27.92)
	≥ 60 y	Seropositivity n/N	19/27	20/27
		(%)	(70.37)	(74.07)
		GMT	8.21	11.75
		(95% CI)	(04.83–13.94)	(06.55–21.12)
	Placebo	Seropositivity n/N	0/11	N/D
		(%)	(0)	(-)
		GMT	0	N/D
		(95% CI)	(0)	(-)
Pseudotyped virus neutralization	Total vaccine	Seropositivity n/N	66/69	66/69
		(%)	(95.65)	(95.65)
		GMT	52.22	41.33
		(95% CI)	(35.12–77.65)	(29.10–56.69)
	18–59 y	Seropositivity n/N	44/45	44/45
		(%)	(97.78)	(97.78)
		GMT	83.74	59.37
		(95% CI)	(51.78–135.4)	(38.08–92.58)
	≥ 60 y	Seropositivity n/N	22/24	22/24
		(%)	(91.67)	(91.67)
		GMT	26.07	22.31
		(95% CI)	(14.91–45.59)	(13.39–37.18)
	Placebo	Seropositivity n/N	0/10	N/D
		(%)	(0)	(-)
		GMT	0	N/D
		(95%CI)	(0)	(-)
Conventional virus neutralization	Total vaccine	Seropositivity n/N	55/72	60/72
		(%)	(76.39)	(83.33)
		GMT	10.10	15.54
		(95% CI)	(7.28–14.01)	(22.18)
	18–59 y	Seropositivity n/N	36/45	38/45
		(%)	(80.0)	(84.44)
		GMT	10.60	14.81
		(95% CI)	(6.92–16.26)	(9.49–23.09)
	≥ 60 y	Seropositivity n/N	19/27	22/27
		(%)	(70.37)	(81.48)
		GMT	9.32	16.84
		(95% CI)	(5.43–15.99)	(8.95–31.67)
	Placebo	Seropositivity n/N	6/11	N/D
		(%)	(54.54)	(-)
		GMT	5.48	N/D
		(95% CI)	(1.84–16.29)	(-)

Timepoints refer to the number of days after the first dose of vaccine or placebo in the schedule.

Abbreviations: CI, confidence interval; GMT, geometric mean titer; N, nucleoprotein; N/D, not determined; S, Spike protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

(Figure 5B and 5D). No changes were detected when stimulating with MP-R alone (Figure 5C). Because MP-CD8A and MP-CD8B were determined in silico to stimulate $CD8^+$ T cells, the expression of AIMs was evaluated on these cells for 21 volunteers. Modest increases in the expression of AIMs were detected for both MP-CD8A and MP-CD8B (Figure 5E and 5F).

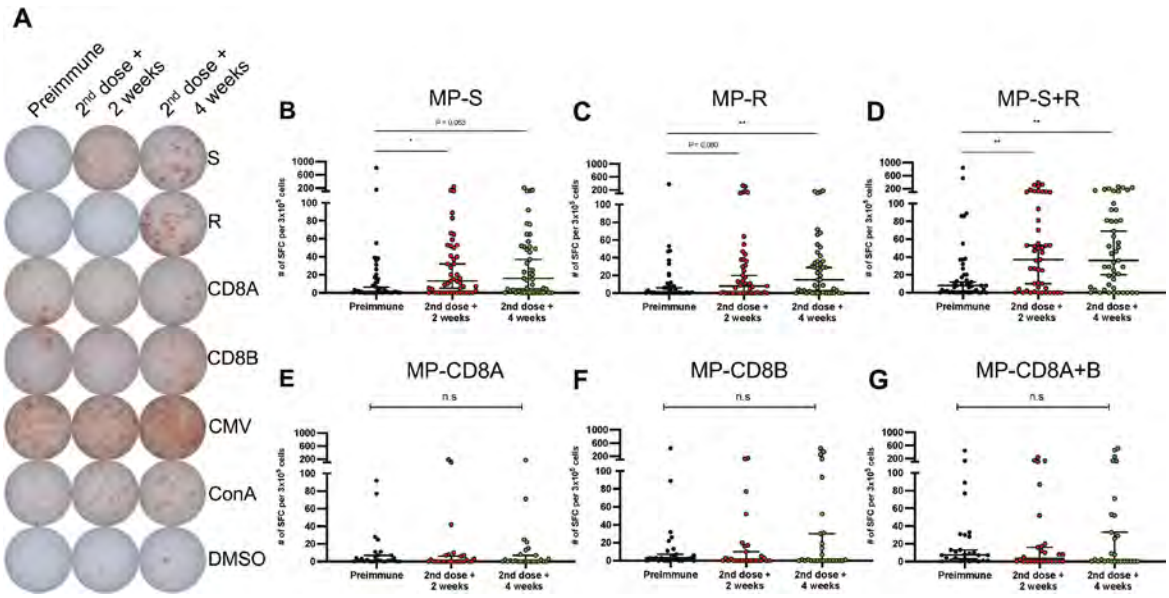


Figure 4. Evaluation of cellular immune response through ELISPOT upon stimulation with Mega Pools of peptides derived from SARS-CoV-2 proteins in volunteers immunized with CoronaVac. Numbers of IFN-γ-secreting cells, determined through ELISPOT as spot forming cells (SFCs) were determined. (*A*) Representative pictures for each stimulus are shown. PBMCs were stimulated with (*B*) MP-S, (*C*) MP-R, (*D*) MP-S + R, (*E*) MP-CD8A, (*F*) MP-CD8B, and (*G*) MP-CD8A + B for 48 h for samples obtained before the first dose, and 2 (second dose + 2 weeks) and 4 weeks (second dose + 4 weeks) after the second dose. A total of 47 volunteers were evaluated for MP-S and MP-R and 27 volunteers for MP-CD8A and MP-CD8B. Data shown represent median ± 95% CI. Statistical differences were evaluated by a Friedman test for repeated measures, followed by a post hoc Dunn test corrected for multiple comparisons against day preimmune samples; n.s. = no statistical differences, $*P < .05$, $**P < .005$. Abbreviations: CI, confidence interval; ELISPOT, enzyme-linked immunospot; IFN, interferon; MP, Mega Pools; PBMC, peripheral blood mononuclear cell; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

No changes were detected for the placebo group (Figure S9). Stimulation with cytomegalovirus and Concanavalin A confirmed the capacity of these cells to express AIMs (Figure S10). Although more volunteers must be evaluated, ELISPOT and flow cytometry results suggest that stimulation with these MPs induces a cellular immune response in volunteers immunized with CoronaVac.

DISCUSSION

This study is a preliminary analysis of a phase 3 clinical trial performed in Chile with CoronaVac, an inactivated SARS-CoV-2 vaccine. We found that 2 doses of CoronaVac, in a 0–14 schedule, were safe and capable of inducing a humoral and cellular immune response in both age groups evaluated (18–59 and ≥60 years), which is in line with the phase 3 trial conducted in Turkey using the same vaccination schedule [21]. However, other studies using CoronaVac support the idea that a vaccination schedule with each dose separated by 4 weeks (0–28) induces better immune responses and shows a better efficacy profile [13]. A phase 2 trial conducted in China with CoronaVac compared both vaccination schedules and reported better immunogenicity in subjects vaccinated with a 0–28 schedule [13]. A recent study evaluating immune responses 6 months after the second dose in volunteers from both vaccination schedules reported higher seropositivity in individuals from the 0–28 schedule [22]. These results are consistent with published data from subjects vaccinated with messenger RNA (mRNA) vaccines, in which higher efficacy has been reported with longer intervals between doses [23, 24]. Therefore, a different immunization schedule considering a booster 4 weeks after the first dose instead of 2 weeks is being tested.

This study has relevant limitations that must be addressed, such as the reduced samples size evaluated for the immunogenicity profile. Also, although the high immunogenicity described here is encouraging, efficacy and death prevention data will be needed to guide the use of this vaccine in clinical and public health settings [13, 14, 20]. It is also important to note that further analyses are required to evaluate the relevance of this vaccine on emerging circulating variants.

Adverse reactions observed were primarily mild and local, which coincides with previous reports with this vaccine. No SAEs were reported for either the vaccine or placebo arm. We detected differences between the age groups in local and systemic AEs, being more frequent in the 18–59 age group than in the ≥60 age group.

Seroconversion rates for S1-RBD-specific IgG and seropositivity of neutralizing antibodies in this study are consistent

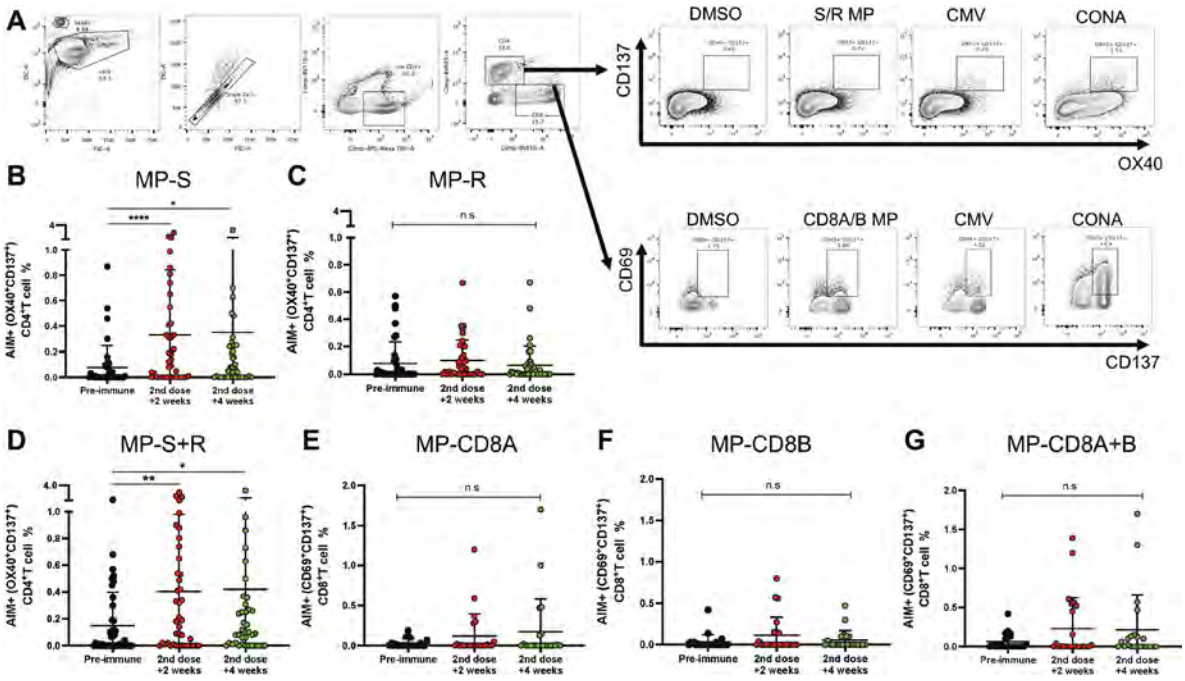


Figure 5. Changes in activation-induced markers (AIMs) expression in T cells through flow cytometry upon stimulation with Mega Pools of peptides derived from SARS-CoV-2 in volunteers immunized with CoronaVac. (*A*) The gating strategy used to evaluate changes in the expression of AIMs upon stimulation of PBMCs is shown. PBMCs were stimulated with (*B*) MP-S, (*C*) MP-R, (*D*) MP-S + R, (*E*) MP-CD8A, (*F*) MP-CD8B, and (*G*) MP-CD8A + B for 24 h for samples obtained before the first dose, and 2 (second dose + 2 weeks) and 4 weeks (second dose + 4 weeks) after the second dose. Changes in the expression of AIMs for $CD4^+$ T cells ($OX40^+$ $CD137^+$) were measured upon stimulation with (*B*) MP-S, (*C*) MP-R, and (*D*) MP-S + R. Changes in the expression of AIMs for $CD8^+$ T cells ($CD69^+$ $CD137^+$) were measured upon stimulation with (*E*) MP-CD8A, (*F*) MP-CD8B, and (*G*) MP-CD8A + B. A total of 43 volunteers were evaluated for MP-S and MP-R and 21 volunteers for MP-CD8A and MP-CD8B. Data shown represent mean ± standard deviation. Statistical differences were evaluated by a Friedman test for repeated measures, followed by a post hoc Dunn test corrected for multiple comparisons against preimmune samples. n.s. = no statistical differences, $^*P < .05$, $^{**}P < .005$, $^{****}P < .0001$. Abbreviations: MP, Mega Pools; PBMC, peripheral blood mononuclear cell; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

with the data reported in the phase 2 trial conducted in China for the same immunization schedule, dose, and age [13]. The geometric median unit values obtained for anti-S1-RBD and anti-N antibodies in this study are somewhat lower than those described for the BNT162b2 (490.17 and 34.40 after the second dose, respectively) and the mRNA-1273 (659.91 and 37.03 after the second dose, respectively) vaccines when using the same international WHO units [25]. Possible differences in these values may be linked to a higher production of antibodies against a single antigen by mRNA vaccines compared with inactivated vaccines, which aim to induce a polyclonal response against several viral proteins [26]. The low production of anti-N antibodies compared with IgG induced against the S1-RBD is not related to the absence of the N protein in CoronaVac. Previous reports indicate that humans naturally infected with SARS-CoV-2 develop antibody responses mainly against the S and N proteins, in somewhat similar levels [12]. However, immunization studies of mice, rats, and nonhuman primates with CoronaVac showed that antibodies induced mainly were directed against the S protein and the S1-RBD, with a reduced number of antibodies against the N protein [12]. This is in line with our findings, suggesting that the enhanced secretion of antibodies against the S protein by CoronaVac, rather than against the N protein, may be playing a role in the protective response.

This is the first time a characterization of the cellular response against proteins other than the S protein of SARS-CoV-2 has been reported in humans immunized with CoronaVac. Unlike previous studies [13], we detected a robust T-cell response upon stimulation of PBMCs with MPs of peptides from S (MP-S). We also evaluated the response elicited upon stimulation with 2 MPs of peptides designed to stimulate a $CD8^+$ T-cell response. Although more volunteers are required to raise more robust conclusions, the results suggest that the $CD8^+$ immune response detected in vaccinated volunteers is not as robust as the $CD4^+$ response. Because increased numbers of IFN-γ secreting cells and reduced amounts of interleukin-4 secreting cells align with a well-balanced Th1 immune response that could lead to virus clearance, immunization with CoronaVac shows promising capacities of inducing an antiviral response in the host. This IFN-γ response has also been sought and observed in other vaccines against SARS-CoV-2, such as the BNT162b1 designed by BioNTech [27] and

the recombinant adenovirus type-5 vectored COVID-19 vaccine designed by CanSino [28].

In summary, immunization with CoronaVac is safe and induces robust humoral and cellular responses, characterized by increased antibody titers against the S1-RBD with neutralizing capacities and the production of T cells specific for several SARS-CoV-2 antigens and were characterized by the secretion of Th1 cytokines.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. Conceptualization: S. M. B., K. A., P. A. G., G. Z., W. M., J. V. G.-A., and A. M. K. Visualization: S. M. B., N. M. S. G., J. A. S., L. F. D., B. M. S., and G. A.P. Methodology: S. M. B., K. A., P. A. G., N. M. S. G., J. A. S., L. F. D., B. M. S., G. A.P., L. A. G., Y. V., M. R., F. M.-G., D. R.-P., C. I., M. U., A. D., C. A. P., R. V. B., G. C.-M., C. C., D. M.-T., F. S., O. P. V., R. F., J. F., J. M., E. R., A. G.-A., A. O.-A., F. V.-E., R. S.-R., D. W., A. S., J. V. G.-A., and A. M. K. Investigation: N. M. S. G., J. A. S., L. F. D., B. M. S., G. A.P., L. A. G., Y. V., M. R., F. M.-G., D. R.-P., C. I., M. U., A. D., C. A. P., R. V. B., G. C.-M., C. C., D. M.-T., F. S., O. P. V., P. D., P. E., D. F., M. G., P. G., P. M.-V., C. M. P., M. P., A. R., R. F., J. F., J. M., E. R., A. G.-A., A. O.-A., F. V.-E., R. S.-R., and D. W. Funding acquisition: A. M. K. Project administration: S. M. B., K. A., P. A. G., G. Z., W. M., J. V. G.-A., and A. M. K. Supervision: S. M. B., K. A., P. A. G., C. I., M. U., J. V. G.-A., and A. M. K. Writing—original draft: S. M. B., P. A. G., N. M. S. G., J. A. S., L. F. D., B. M. S., G. A.P., and A. M. K. Writing—review and editing: S. M. B., K. A., P. A. G., N. M. S. G., J. A. S., L. F. D., B. M. S., G. A.P., L. A. G., Y. V., M. R., F. M.-G., D. R.-P., C. I., M. U., A. D., C. A. P., R. V. B., G. C.-M., C. C., D. M.-T., F. S., O. P. V., P. D., P. E., D. F., M. G., P. G., P. M.-V., C. M. P., M. P., A. R., R. F., J. F., J. M., E. R., A. G.-A., A. O.-A., F. V.-E., R. S.-R., D. W., A. S., J. V. G.-A., and A. M. K.

Acknowledgments. The authors thank the Ministry of Health, Government of Chile; Ministry of Science, Technology, Knowledge, and Innovation, Government of Chile; and The Ministry of Foreign Affairs, Government of Chile and the Chilean Public Health Institute (ISP). They also thank PATH for their support and sharing the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin. We are grateful to Rami Scharf, Jessica White, Jorge Flores, and Miren Iturriza-Gomara from PATH for their support on experimental design and discussion; Alex Cabrera and Sergio Bustos from the Flow Cytometry Facility at Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile for support with flow cytometry; and Sebastián Silva from the same Faculty for support with statistical analyses. The authors also thank the Vice Presidency of Research (VRI), the Direction of Technology Transfer and Development (DTD), and the Legal Affairs Department (DAJ) of the Pontificia Universidad Católica de Chile and are also grateful to the Administrative Directions of the School of Biological Sciences and the School of Medicine of the Pontificia Universidad Católica de Chile for their administrative support. Special thanks to the members of the independent data safety monitoring committee (members in the supplementary Appendix) for their oversight, and the subjects enrolled in the study for their participation and commitment to this trial. Members of the CoronaVac03CL Study Team are listed in the supplemental Appendix. All data are available in the main text or the supplementary information and upon request to the corresponding authors.

Financial support. The Ministry of Health, Government of Chile, supported the funding of the CoronaVac03CL Study; The Confederation of Production and Commerce (CPC), Chile, supported the funding of the CoronaVac03CL Study; The Millennium Institute on Immunology and Immunotherapy, ANID—Millennium Science Initiative Program ICN09_016 (former P09/016-F) supports S. M. B., K.A., P. A. G., and A. M. K.; NIH contracts and 75N9301900065 awarded to A. S. and D. W.; The Innovation Fund for Competitiveness FIC-R 2017 (BIP Code: 30488811–0) supports S. M. B., P. A. G., and A. M. K.; FONDECYT grants Nº 1190156 awarded to R. S. R. and Nº 1180798 awarded to F. V. E.; SINOVAC Life Science Co contributed to this study with the investigational vaccine and placebo, and experimental reagents.

Potential conflicts of interest. Z. G. and M. W. are SINOVAC employees and contributed to the conceptualization of the study (clinical protocol and electronic case report form design) and did not participate in the analysis or interpretation of the data presented in the manuscript. P. M. V. reports funding and study materials from the trial sponsor, during the conduct of the study; reports support from Agencia Nacional de Investigación y Desarrollo (Fondecyt regular); reports being a member of scientific advisory committee for COVID Vaccines, Ministry of Science, technology, knowledge, and innovation, outside of the submitted work. A. G. A. reports receiving ANID/Conicyt Chile grant no. 21181508 (doctoral fellowship awarded by the Agencia Nacional de Investigación y Desarrollo [ANID] Chile), outside the submitted work. F. V.-E. reports receiving Fondecyt 1180798 – 1211547 (National Research and Development Agency), during the conduct of the study; served as Chilean Microbiology Society President 2021–2022. RS-R reports receiving research grant (Nº 1190156) from the FONDECYT Program (funds from this research grant were employed in neutralization assays involving the pseudotyped virus) (ANID Chile), during the conduct of the study. D. W. reports receiving NIH contract no. 75N9301900065 (D. W., A. S.).; reports that LJI has filed for patent protection for various aspects of T-cell epitope and vaccine design work. A. S. reports receiving NIH contract no. 75N9301900065 (D. W., A. S.); A. S. is a paid consultant for Gritstone, Flow Pharma, Arcturus, Immunoscape, CellCarta, OxfordImmunotech and Avalia; LJI has filed for patent protection for various aspects of T-cell epitope and vaccine design work. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med **2020**; 382:727–733. Available at: http://www.ncbi.nlm.nih.gov/pubmed/31978945.
2. Kim D, Lee J-Y, Yang J-S, Kim JW, Kim VN, Chang H. The architecture of SARS-CoV-2 transcriptome. Cell **2020**; 181:914–21.e10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/32330414.
3. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature **2020**; 579:270–3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/32015507.
4. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis **2020**; 20:533–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/32087114.
5. Perez-Saez J, Lauer SA, Kaiser L, et al. Serology-informed estimates of SARS-CoV-2 infection fatality risk in Geneva, Switzerland. Lancet Infect Dis **2020**; Available at: http://www.ncbi.nlm.nih.gov/pubmed/32679085.
6. O'Driscoll M, Ribeiro Dos Santos G, Wang L, et al. Age-specific mortality and immunity patterns of SARS-CoV-2. Nature **2021**; 590:140–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/33137809.
7. World Health Organization. Draft landscape of COVID-19 candidate vaccines. **2020**. Available at: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines. Accessed 6 October 2021.
8. Krammer F. SARS-CoV-2 vaccines in development. Nature **2020**; 586:516–27. Available at: http://www.nature.com/articles/s41586-020-2798-3.
9. Stern PL. Key steps in vaccine development. Ann Allergy Asthma Immunol **2020**; 125:17–27. Available at: http://www.ncbi.nlm.nih.gov/pubmed/32044451.
10. Al Kaabi N, Zhang Y, Xia S, et al. Effect of 2 inactivated SARS-CoV-2 vaccines on symptomatic COVID-19 infection in adults: a randomized clinical trial. JAMA **2021**; 326:35–45. Available at: http://www.ncbi.nlm.nih.gov/pubmed/34037666.
11. Li Y-D, Chi W-Y, Su J-H, Ferrall L, Hung C-F, Wu T-C. Coronavirus vaccine development: from SARS and MERS to COVID-19. J Biomed Sci **2020**; 27:104. Available at: http://www.ncbi.nlm.nih.gov/pubmed/33341119.

12. Gao Q, Bao L, Mao H, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science **2020**; 369:77–81. Available at: http://www.ncbi.nlm.nih.gov/pubmed/32376603.
13. Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis **2021**; 21:181–92. Available at: http://www.ncbi.nlm.nih.gov/pubmed/33217362.
14. Wu Z, Hu Y, Xu M, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis **2021**; Available at: http://www.ncbi.nlm.nih.gov/pubmed/33548194.
15. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA **2013**; 310:2191–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24141714.
16. Beltrán-Pavez C, Riquelme-Barrios S, Oyarzún-Arrau A, et al. Insights into neutralizing antibody responses in individuals exposed to SARS-CoV-2 in Chile. Sci Adv **2021**; 7:eabe6855. Available at: http://www.ncbi.nlm.nih.gov/pubmed/33579701.
17. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell **2020**; 181:1489–501.e15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/32473127.
18. Departamento de Epidemiología G de C. Reporte de la circulación de variantes de SARS-CoV-2 en Chile. Santiago: 2021. Available at: https://www.minsal.cl/wp-content/uploads/2021/02/Reporte-circulacion-variantes-26-02-21.pdf. Accessed 14 August 2021.
19. WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A minimal common outcome measure set for COVID-19 clinical research. Lancet Infect Dis **2020**; 20:e192–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/32539990.
20. He Q, Mao Q, Zhang J, et al. COVID-19 vaccines: current understanding on immunogenicity, safety, and further considerations. Front Immunol **2021**; 12:669339. Available at: http://www.ncbi.nlm.nih.gov/pubmed/33912196.
21. Tanriover MD, Doğanay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. Lancet **2021**; 398:213–22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/34246358.
22. Pan H, Wu Q, Zeng G, et al. Immunogenicity and safety of a third dose, and immune persistence of CoronaVac vaccine in healthy adults aged 18-59 years: interim results from a double-blind, randomized, placebo-controlled phase 2 clinical trial. medRxiv **2021**; 2021.07.23.21261026. Available at: http://medrxiv.org/content/early/2021/07/25/2021.07.23.21261026.abstract.
23. Voysey M, Costa Clemens SA, Madhi SA, et al. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. Lancet **2021**; 397:881–91. Available at: http://www.ncbi.nlm.nih.gov/pubmed/33617777.
24. Parry H, Bruton R, Stephens C, et al. Extended interval BNT162b2 vaccination enhances peak antibody generation in older people. medRxiv **2021**;2021.05.15.21257017. Available at: http://medrxiv.org/content/early/2021/05/17/2021.05.15.21257017.abstract.
25. Kung Y-A, Huang C-G, Huang S-Y, et al. Antibody titers measured by commercial assays are correlated with neutralizing antibody titers calibrated by international standards. medRxiv **2021**;2021.07.16.21260618. Available at: http://medrxiv.org/content/early/2021/07/22/2021.07.16.21260618.abstract.
26. Kyriakidis NC, López-Cortés A, González EV, Grimaldos AB, Prado EO. SARS-CoV-2 vaccines strategies: a comprehensive review of phase 3 candidates. NPJ Vaccines **2021**; 6:28. Available at: http://www.ncbi.nlm.nih.gov/pubmed/33619260.
27. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature **2020**; 586:594–9. Available at: http://www.nature.com/articles/s41586-020-2814-7.
28. Zhu F-C, Li Y-H, Guan X-H, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet **2020**; 395:1845–54. Available at: http://www.ncbi.nlm.nih.gov/pubmed/32450106.

1.15. CoronaVac dobra número de anticorpos neutralizantes e aumenta IgG em 4,4 vezes em quem já teve Covid-19

Um estudo feito por pesquisadores da Universidade Médica de Chongqing, na China, com 85 pacientes recuperados de Covid-19 indica que a CoronaVac, vacina do Butantan e da farmacêutica Sinovac, é capaz de dobrar a quantidade de anticorpos neutralizantes e multiplicar em 4,4 vezes o nível de imunoglobulina IgG em quem já teve a doença. Os resultados preliminares foram divulgados na Cell Discovery, publicação que faz parte do grupo britânico Nature, no artigo Humoral responses in naive or SARS-CoV-2 experienced individuals vaccinated with an inactivated vaccine.

Os participantes da pesquisa tinham entre três e 84 anos e haviam se contaminado de Covid-19, em sua maioria, no início de 2020. Os pesquisadores aferiram os níveis de imunoglobulinas e de anticorpos neutralizantes nos pacientes convalescentes e selecionaram os cinco que apresentaram individualmente os menores indicadores ao final de 12 meses. Eles receberam duas doses de CoronaVac com intervalo de 21 dias.

O nível de anticorpos neutralizantes (que protegem contra uma eventual reinfecção pelo SARS-CoV-2) entre as pessoas que tiveram Covid-19, que era de 36 um dia antes da primeira dose, foi subindo até atingir 108 duas semanas após a segunda dose. No grupo controle, esse indicador alcançou 56 – ou seja, a quantidade de anticorpos neutralizantes gerados pela vacina em quem já teve Covid-19 foi o dobro na comparação com quem não havia tido a doença.

Entre os convalescentes, o nível de anticorpos IgG, que era de 3,68 um dia antes da vacina, subiu para 47,74 duas semanas após a segunda dose de CoronaVac. É uma quantidade 4,4 vezes superior ao nível de 10,81 detectado no grupo controle. O IgG se relaciona à imunidade humoral, processo de defesa do organismo no qual atuam as imunoglobulinas encontradas na corrente sanguínea. A resposta imune humoral é crítica para o combate ao SARS-CoV-2 e também desempenha papel fundamental na prevenção de reinfecção viral.

Ao longo dos 12 meses de acompanhamento dos pacientes, os níveis dos anticorpos neutralizantes diminuíram de 631 no final do primeiro mês para 84 no último mês. No caso da imunoglobulina IgG, o indicador caiu de 28,6 para 7,2 no mesmo período.

Os resultados da pesquisa sugerem que a CoronaVac estimula a memória humoral dos pacientes convalescentes, acelerando a produção de anticorpos neutralizantes e seu nível de circulação na corrente sanguínea.

Publicado em: 17/08/2021

Peng et al. *Cell Discovery* (2021)7:68
https://doi.org/10.1038/s41421-021-00311-z

Cell Discovery
www.nature.com/celldisc

CORRESPONDENCE Open Access

Humoral responses in naive or SARS-CoV-2 experienced individuals vaccinated with an inactivated vaccine

Pai Peng[1], Hai-jun Deng[1], Jie Hu[1], Xiao-yu Wei[2], Jian-jiang Xue[3], Ting-ting Li[4], Liang Fang[2], Bei-zhong Liu[2], Ai-shun Jin[4], Feng-li Xu[1], Kang Wu[1], Quan-xin Long[1], Juan Chen[1], Kai Wang[1]✉, Ni Tang[1]✉ and Ai-long Huang[1]✉

Dear Editor,

The humoral immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is critical for the clearance of the virus and also plays a key role for the prevention of viral reinfection. It has been extensively reported that antibody response to SARS-CoV-2 tends to be diminished in course of time[1–3]. Thus, the durability of the protective immune response in coronavirus disease-2019 (COVID-19) recovered patients is of great interest. There is increasing appreciation of the key role that immunological memory plays in durable protective immunity after infections or vaccinations, even with lower antibody titers[4,5]. Inactivated vaccines as a conventional vaccine development have been shown to be effective among other viruses[6]. It has raised concern about the impact of prior infection by SARS-CoV-2 on the immune response induced by inactivated vaccines. For these reasons, we examined the humoral immunity in convalescent patients for 12 months postsymptom onset (PSO) and evaluated the immune response elicited by an inactivated vaccine in naive or COVID-19 recovered individuals.

170 blood samples from a follow-up cohort of 85 COVID-19 patients were collected over a 12-month period PSO (Supplementary Fig. S1a). Participants with 57.6% male and 42.4% female aged from 3 to 84 (median: 48 years) were enrolled (Supplementary Table S1). After the measurement of neutralizing antibodies (NAbs), five participants with low NAb titers were given two injections of CoronaVac vaccine (developed by Sinovac Life Sciences, China) 21 days apart for the study of immunological memory response. Meanwhile, 19 healthy individuals were recruited as the control group (Supplementary Fig. S1b, Table S2).

Anti-SARS-CoV-2 spike (anti-S) IgG/IgM/IgA and NAb titers were measured with previously described MCLIA kits and pseudovirus-based neutralization assay. Anti-S IgG and NAbs were still detectable in 95.5% (42 of 44) and 93.2% (41 of 44) serum samples, respectively, at 12 months PSO (Fig. 1a). Correlation between anti-S IgG levels and Nab titers ($r = 0.64$, $p = 5.8e{-}21$) was shown over the study period (Supplementary Fig. S2a). Nevertheless, during the 12-month follow-up visit in the COVID-19 recovery cohort, anti-S IgG/IgM/IgA and NAb titers represented a sustained decline (Fig. 1a, Supplementary Fig. S2b, c). For the neutralizing antibodies, median of NAb titers decreased from 631 at Month 1 to 604 at Month 3, to 134 at Month 8 and to 84 at Month 12. For the IgG antibodies, the median of signal-to-cutoff ratio (S/CO) dropped from 28.6 at Month 1 to 27.7 at Month 3, 11.5 at Month 8 and 7.2 at Month 12. At Month 12, the levels of specific antibodies were much lower than the levels at Month 1 (82.8%, 96.4%, and 89.4% decrease for IgG, IgM, and IgA antibodies, respectively). In addition, a longitudinal study was observed among nine participants provided samples at all follow-up time points. In spite of a general decline in humoral immune response, the dynamic changes showed significant variation between anti-S IgG/IgM/IgA antibodies and NAbs

Correspondence: Kai Wang (wangkai@cqmu.edu.cn) or Ni Tang (nitang@cqmu.edu.cn) or Ai-long Huang (ahuang@cqmu.edu.cn)
[1]Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Institute for Viral Hepatitis, Department of Infectious Diseases, the Second Affiliated Hospital, Chongqing Medical University, Chongqing, China
[2]Yong-Chuan Hospital, Chongqing Medical University, Chongqing, China
Full list of author information is available at the end of the article
These authors contributed equally: Pai Peng, Hai-jun Deng, Jie Hu

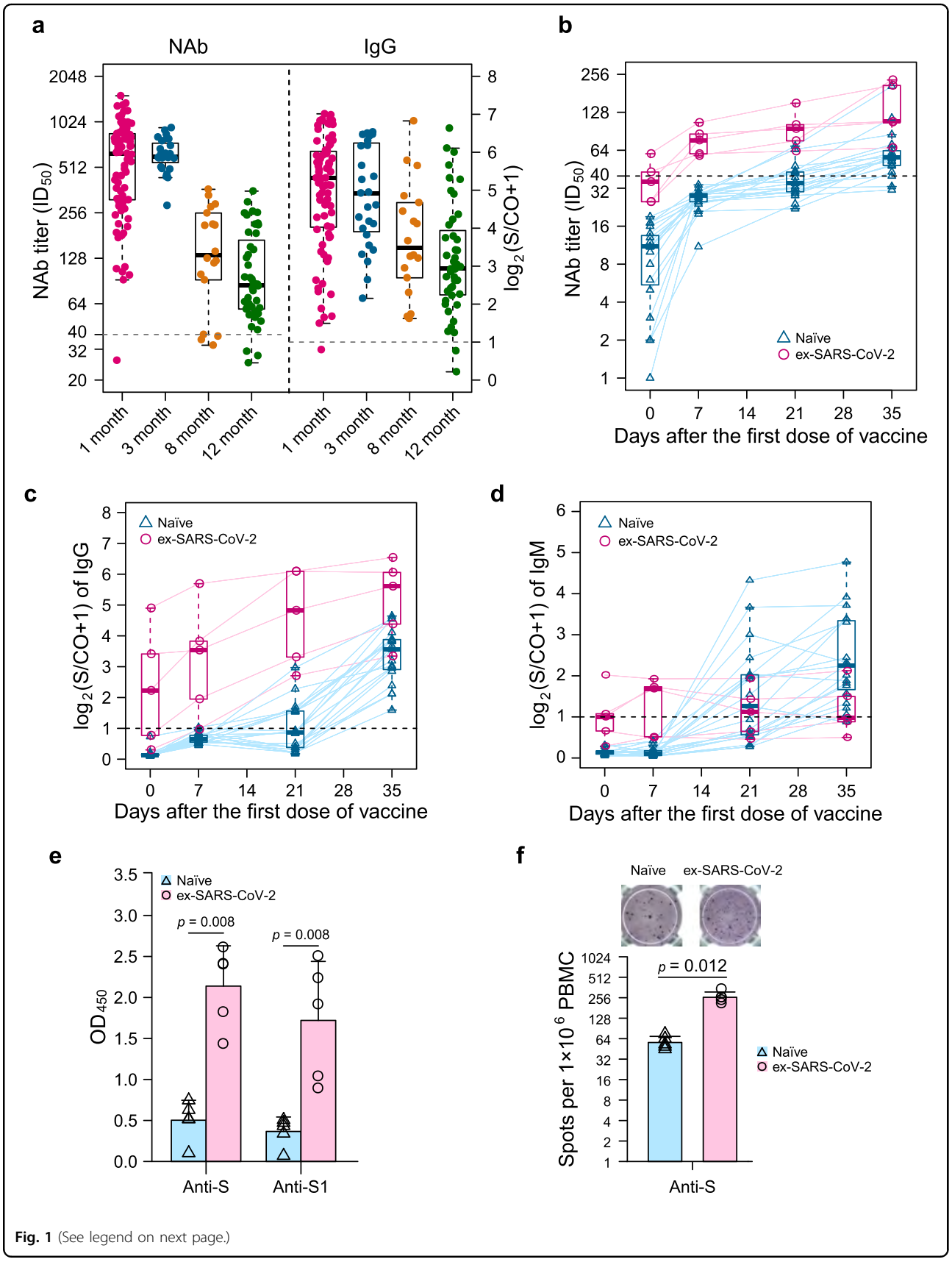


Fig. 1 (See legend on next page.)

(see figure on previous page)

Fig. 1 Immunological memory response of COVID-19 recovered individuals elicited by an inactivated vaccine at 12 months PSO. a Dynamic changes of antibody response in a cohort of COVID-19 recovered individuals from 1 to 12 months. SARS-CoV-2 specific IgG/IgM/IgA and NAb titers were measured with previously described MCLIA kits and pseudovirus-based neutralization assay. Medians (interquartile range, IQR) are shown. The NAb titers were calculated as 50% inhibitory dose (ID_{50}) and the limit of detection (LOD) was 40; the signal to cut-off ratio (S/CO) of IgG/IgM/IgA above 1 was considered as positive. NAb titers (**b**), IgG (**c**), and IgM (**d**) levels of two cohort in which COVID-19 convalescent individuals or healthy participants were injected by two-dose inactivated vaccine CoronaVac; **e**, **f** the status of SARS-CoV-2 specific memory B cells in COVID-19 recovered individuals and naive individuals. Enzyme-linked immunosorbent assay (ELISA) (**e**) was performed to detected anti-S, anti-S1 IgG secreted by memory B cells and enzyme-linked immunosorbent spot assay (ELISpot) (**f**) was performed to analyze the number of antibody-secreting cells. OD denotes optical density, S spike protein and S1 fragment of spike glycoprotein. Empty triangles with red and empty circles with blue indicate healthy individuals and SARS-CoV-2 experienced individuals, respectively; the horizontal dashed lines denote the lower LOD. In **a**–**d**, boxes denote the median, first and third quartiles, while the whiskers show ×1·5 interquartile range (IQR) of antibody levels. In **e**, **f**, boxes and error bars denote mean ± standard deviation. Statistical analysis was performed with the use of the two-tailed, nonparametric Mann–Whitney *U* test.

(Supplementary Fig. S2d–g). Both IgM and IgA levels in 7 of 9 individuals reached peak at 1 month PSO and fell below the positive threshold thereafter. By contrast, IgG and NAbs decreased slowly and remains 100% (9/9) and 78% (7/9) positive at 12 months PSO.

Blood samples from two vaccination cohorts were collected pre-vaccination (day 0, the day before the first dose of vaccine) and 7, 21, 35 days after the first dose of vaccine (Supplementary Fig. S1b). The evaluation of immunological memory induced by the inactivated vaccine was performed by detection of specific antibodies and antibody-secreting memory B cells among participants. NAbs were detective only in COVID-19 recovered group within 7 days after the first dose of vaccine (median of NAb titers 36 on Day 0; 77 on Day 7; 95 on Day 21; and 108 on Day 35) (Fig. 1b). The median NAbs titer was 56 in the naive group 35 days after the first dose of vaccine. Due to the previous presence of SARS-CoV-2 specific antibodies, the majority of COVID-19 recovered individuals had detectable IgG from pre-vaccination to post-vaccination (median S/CO value before vaccination, 3.68; and 10.59, 27.33, and 47.74 on Day 7, 21, and 35 after vaccination, respectively) (Fig. 1c). In the naive group, anti-S IgG was detected with lower values than COVID-19 recovered individuals over 35 days after the first dose of vaccine (median S/CO value before vaccination, 0.10; and 0.57, 0.83, and 10.81 on Day 7, 21, and 35 after the first dose of vaccine, respectively). IgG levels of COVID-19 recovered individuals were 4.4 times that of naive individuals at Day 35 (median S/CO value, 47.74 vs 10.81). Interestingly, IgM titers increased over time in naive group, while no substantial changes displayed in COVID-19 recovered group (Fig. 1d). Furthermore, IgA of both groups remained at a low level, even staying below the positive threshold (Supplementary Fig. S3).

To further understand higher humoral response in COVID-19 recovered individuals after vaccination, SARS-CoV-2 specific memory B cells differentiated from peripheral blood mononuclear cells of 5 SARS-CoV-2 experienced and naive individuals before vaccination were determined by enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunosorbent spot assay (ELISpot). As expected, specific anti-S, anti-S1 fragment of spike glycoprotein (anti-S1) IgG and the number of anti-S IgG antibody-secreting cells presented higher levels in SARS-CoV-2 experienced group than the naive group (Fig. 1e, f).

Our findings demonstrated that anti-S IgG, IgM, IgA and NAb titers declined gradually over 1 year in patients infected with SARS-CoV-2. Even though antibody response of most participants remained detectable, the drop of more than 80% were shown in anti-S IgG, IgM, IgA, and NAb titers. To evaluate the duration of protective immunity against SARS-CoV-2, further surveillance is needed. Moreover, our results suggest that immunological memory mediated by an inactivated vaccine could recall higher response of IgG and NAb in COVID-19 recovered individuals with low NAb titers than in naive persons at 12 months PSO. After infection, SARS-CoV-2 specific memory B cells secreting antibody increased significantly in COVID-19 recovered individuals compared to healthy controls. It should be pointed out that maybe due to the cross-activity between SARS-CoV-2 and seasonal coronaviruses, SARS-CoV-2 S and S1-specific antibodies secreted by memory B cells were detected at baseline in naive persons[7].

Compared to our data, rapid immune response elicited by a single mRNA vaccine dose was showed in several SARS-CoV-2 recovery cohorts vaccinated by mRNA-based vaccines[8–11]. Further investigation is needed to answer the necessity of vaccination for SARS-CoV-2 experienced individuals, and to answer whether the immune response provides effective protection from reinfection in this special group, especially for SARS-CoV-2 variants.

The main limitation of this study is the small sample size and relatively short period for the observation of vaccination cohorts. Even though our data provided a hint about the role of memory B cell response in humoral response after vaccination or reinfection, a deeper investigation carried out by flow cytometry will be needed. An inactivated

virus vaccine including all components of SARS-CoV-2 might provide the distinct benefit to boost T-cell response against other SARS-CoV-2 proteins, but T-cell immunity was not investigated in our study.

Our results reveal the durability of immunological response 1 year after natural SARS-CoV-2 infection and the benefit from inactivated vaccines for COVID-19 recovered individuals. It provides more information about immunological characteristics of SARS-CoV-2 inactivated vaccines, thus will contribute to the development of vaccines and the new strategies of vaccination.

Acknowledgements
We thank Dr. Cheng-yong Yang for critical review of the manuscript. We acknowledge the arrangement of blood sample collection from Yongchuan Hospital Affiliated to Chongqing Medical University (Chongqing, China) and all participants involved in the study. This work had received funding support from the Natural Science Foundation Project of Chongqing (cstc2019jscx-dxwtBX0019), the Emergency Project from the Science & Technology Commission of Chongqing (cstc2020jscx-dxwtB0050, cstc2020jscx-fyzx0053), the Emergency Project for Novel Coronavirus Pneumonia from the Chongqing Medical University (CQMUNCP0302), a Major National Science & Technology Program grant (2017ZX10202203) from the Science & Technology Commission of China, Kuanren Talents Program of the second affiliated hospital of Chongqing Medical University, China Postdoctoral Science Foundation (2021M693924), and Chongqing Postdoctoral Science Special Foundation (2010010005216630).

Author details
[1]Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Institute for Viral Hepatitis, Department of Infectious Diseases, the Second Affiliated Hospital, Chongqing Medical University, Chongqing, China. [2]Yong-Chuan Hospital, Chongqing Medical University, Chongqing, China. [3]University-Town Hospital of Chongqing Medical University, Chongqing, China. [4]Department of Immunology, College of Basic Medicine, Chongqing Medical University, Chongqing, China

Author contributions
P.P. participated in data curation, formal analysis, funding acquisition, investigation, validation, and writing-original draft; J.H., F.L.X., K. Wu. participated in data curation, formal analysis, investigation, and methodology; H.J.D. participated in software, visualization, and writing-review and editing; J.C., Q.X.L., X.Y.W. and B.Z.L. participated in resources; J.J.X., T.T.L., A.S.J. participated in methodology and resources; K. Wang., N.T. and A.L.H. participated in conceptualization, formal analysis, funding acquisition, project administration, supervision, validation and writing-review and editing.

Conflict of interest
The authors declare no competing interests.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41421-021-00311-z.

Received: 25 April 2021 Accepted: 11 July 2021
Published online: 17 August 2021

References

1. Long, Q.-X. et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat. Med.* **26**, 845–848 (2020).
2. Wang, K. et al. Longitudinal dynamics of the neutralizing antibody response to SARS-CoV-2 infection. *Clin. Infect. Dis.* https://doi.org/10.1093/cid/ciaa1143 (2020).
3. Lau, E. H. Y. et al. Neutralizing antibody titres in SARS-CoV-2 infections. *Nat. Commun.* **12**, 63 (2021).
4. Gaebler, C. et al. Evolution of antibody immunity to SARS-CoV-2. *Nature* **591**, 639–644 (2021).
5. Dan, J. M. et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* **371**, eabf4063 (2021).
6. Jackson, M. L. et al. Influenza vaccine effectiveness in the United States during the 2015-2016 season. *N. Engl. J. Med.* **377**, 534–543 (2017).
7. Song, G. et al. Cross-reactive serum and memory B-cell responses to spike protein in SARS-CoV-2 and endemic coronavirus infection. *Nat. Commun.* **12**, 2938 (2021).
8. Krammer, F. et al. Antibody responses in seropositive persons after a single dose of SARS-CoV-2 mRNA vaccine. *N. Engl. J. Med.* **384**, 1372–1374 (2021).
9. Ebinger, J. E. et al. Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2. *Nat. Med.* **27**, 981–984 (2021).
10. Bradley, T. et al. Antibody responses after a single dose of SARS-CoV-2 mRNA vaccine. *N. Engl. J. Med.* **384**, 1959–1961 (2021).
11. Manisty, C. et al. Antibody response to first BNT162b2 dose in previously SARS-CoV-2-infected individuals. *Lancet* **397**, 1057–1058 (2021).

1.16. CoronaVac é segura, bem aceita pelo organismo e tem eficácia de 83,5%, de acordo com estudo clínico turco

Assim como já havia sido confirmado pelos ensaios clínicos de fase 3 conduzidos ao longo de 2020 no Brasil para avaliar a eficácia da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, um estudo da Universidade Hacettepe, com sede em Ancara, na Turquia, mostrou que o imunizante é 83,5% eficaz contra o SARS-CoV-2, além de ser seguro e bem tolerado pelo organismo. A pesquisa foi publicada na revista científica The Lancet e na Biblioteca Nacional de Medicina dos Estados Unidos, a maior biblioteca médica do mundo.

O estudo de fase 3, randomizado e duplo-cego, contou com a participação de 10.218 pessoas e foi feito entre 14 de setembro de 2020 e 5 de janeiro de 2021. Os voluntários foram avaliados sete, 14 e 28 dias depois de tomar cada uma das duas doses. Durante o acompanhamento médio de 43 dias, nove casos sintomáticos de Covid-19 foram confirmados no grupo que tomou a vacina e 32 casos foram relatados no grupo que tomou placebo. A CoronaVac preveniu hospitalizações em todos os voluntários, na comparação com os seis do grupo placebo. Não houve mortes nem no grupo que tomou a vacina e nem no grupo placebo.

Além disso, a CoronaVac induziu anticorpos em 89,7% dos participantes. Destes, 92% também produziram níveis protetores de anticorpos neutralizantes pelo menos 14 dias após a segunda dose da vacina.

O artigo destaca ainda que a vacina mostrou um perfil de segurança satisfatório, sem eventos adversos de grau 4 durante o período do estudo. A maioria dos efeitos adversos foi de grau 1 e ocorreu até sete dias após a injeção. A incidência total foi baixa (18,9%), e o principal sintoma foi fadiga.

"Nossos resultados mostram que a CoronaVac tem boa eficácia contra infecção sintomática por SARS-CoV-2 e Covid-19 grave com um perfil de segurança muito bom em uma população de 18 a 59 anos", afirmaram os autores do artigo. "A tolerabilidade da CoronaVac neste estudo foi excelente e a incidência de eventos adversos foi baixa."

Participaram do estudo voluntários de diferentes grupos de risco e ocupação, tornando os resultados bem próximos ao contexto do mundo real. Receberam a vacina 6.646 pessoas, sendo que 3.568 voluntários tomaram placebo (substância ou tratamento sem um princípio ativo, como uma injeção de soro fisiológico). Do total de participantes, 57,8% eram homens e 42,24% mulheres, todos entre 18 e 59 anos. Desse grupo, 3.675 pessoas eram profissionais de saúde e 1.463 eram obesos. E entre todos os participantes, 6.217 tinham algum tipo de comorbidade – a maioria relatou ter hipertensão.

O ensaio clínico de fase 3 realizado no Brasil pelo Butantan envolveu 16 centros de pesquisa científica em sete estados e no Distrito Federal. O teste duplo cego envolveu 12,5 mil profissionais de saúde, e obteve 62,3% de eficácia global em casos leves, moderados ou graves, num espaço de 21 dias ou mais entre as duas doses.

Publicado em: 8/07/2021

Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey

Mine Durusu Tanriover, Hamdi Levent Doğanay*, Murat Akova*, Hatice Rahmet Güner, Alpay Azap, Sıla Akhan, Şükran Köse, Fatma Şebnem Erdinç, Emin Halis Akalın, Ömer Fehmi Tabak, Hüsnü Pullukçu, Özgür Batum, Serap Şimşek Yavuz, Özge Turhan, Mustafa Taner Yıldırmak, İftihar Köksal, Yeşim Taşova, Volkan Korten, Gürdal Yılmaz, Mustafa Kemal Çelen, Sedat Altın, İlhami Çelik, Yaşar Bayındır, İlkay Karaoğlan, Aydın Yılmaz, Aykut Özkul, Hazal Gür, Serhat Unal*, and the CoronaVac Study Group†*

Summary

Background CoronaVac, an inactivated whole-virion SARS-CoV-2 vaccine, has been shown to be well tolerated with a good safety profile in individuals aged 18 years and older in phase 1/2 trials, and provided a good humoral response against SARS-CoV-2. We present the interim efficacy and safety results of a phase 3 clinical trial of CoronaVac in Turkey.

Methods This was a double-blind, randomised, placebo-controlled phase 3 trial. Volunteers aged 18–59 years with no history of COVID-19 and with negative PCR and antibody test results for SARS-CoV-2 were enrolled at 24 centres in Turkey. Exclusion criteria included (but were not limited to) immunosuppressive therapy (including steroids) within the past 6 months, bleeding disorders, asplenia, and receipt of any blood products or immunoglobulins within the past 3 months. The K1 cohort consisted of health-care workers (randomised in a 1:1 ratio), and individuals other than health-care workers were also recruited into the K2 cohort (randomised in a 2:1 ratio) using an interactive web response system. The study vaccine was 3 μg inactivated SARS-CoV-2 virion adsorbed to aluminium hydroxide in a 0·5 mL aqueous suspension. Participants received either vaccine or placebo (consisting of all vaccine components except inactivated virus) intramuscularly on days 0 and 14. The primary efficacy outcome was the prevention of PCR-confirmed symptomatic COVID-19 at least 14 days after the second dose in the per protocol population. Safety analyses were done in the intention-to-treat population. This study is registered with ClinicalTrials.gov (NCT04582344) and is active but no longer recruiting.

Findings Among 11 303 volunteers screened between Sept 14, 2020, and Jan 5, 2021, 10 218 were randomly allocated. After exclusion of four participants from the vaccine group because of protocol deviations, the intention-to-treat group consisted of 10 214 participants (6646 [65·1%] in the vaccine group and 3568 [34·9%] in the placebo group) and the per protocol group consisted of 10 029 participants (6559 [65·4%] and 3470 [34·6%]) who received two doses of vaccine or placebo. During a median follow-up period of 43 days (IQR 36–48), nine cases of PCR-confirmed symptomatic COVID-19 were reported in the vaccine group (31·7 cases [14·6–59·3] per 1000 person-years) and 32 cases were reported in the placebo group (192·3 cases [135·7–261·1] per 1000 person-years) 14 days or more after the second dose, yielding a vaccine efficacy of 83·5% (95% CI 65·4–92·1; p<0·0001). The frequencies of any adverse events were 1259 (18·9%) in the vaccine group and 603 (16·9%) in the placebo group (p=0·0108) with no fatalities or grade 4 adverse events. The most common systemic adverse event was fatigue (546 [8·2%] participants in the vaccine group and 248 [7·0%] the placebo group, p=0·0228). Injection-site pain was the most frequent local adverse event (157 [2·4%] in the vaccine group and 40 [1·1%] in the placebo group, p<0·0001).

Interpretation CoronaVac has high efficacy against PCR-confirmed symptomatic COVID-19 with a good safety and tolerability profile.

Funding Turkish Health Institutes Association.

Lancet 2021; 398: 213–22

Published Online July 8, 2021 https://doi.org/10.1016/S0140-6736(21)01429-X

See Comment page 186

*Contributed equally

†Members of the CoronaVac Study Group and all participating sites are listed in the appendix (pp 15–16)

Department of Internal Medicine (Prof M D Tanriover MD), **Department of Infectious Diseases and Clinical Microbiology** (Prof M Akova MD, Prof S Unal MD), **and Department of Medical Microbiology** (H Gür MSc), **Hacettepe University School of Medicine, Ankara, Turkey; Hacettepe University Vaccine Institute, Ankara, Turkey** (Prof M D Tanriover, Prof M Akova, Prof S Unal); **Department of Gastroenterology, Turkish Republic Ministry of Health, İstanbul Provincial Health Directorate, University of Health Sciences İstanbul Ümraniye Training and Research Hospital, İstanbul, Turkey** (Prof H L Doğanay MD); **Department of Infectious Diseases and Clinical Microbiology, Ankara Yıldırım Beyazıt University, Ankara City Hospital, Ankara, Turkey** (Prof H R Güner MD); **Department of Infectious Diseases and Clinical Microbiology, Ankara University School of Medicine, Ankara, Turkey** (Prof A Azap MD); **Department of Infectious Diseases and Clinical Microbiology, Kocaeli University School of Medicine, Kocaeli, Turkey** (Prof S Akhan MD); **Department of Infectious Diseases, Turkish**

Introduction

The COVID-19 pandemic continues to affect individuals and populations, magnifying socioeconomic and health inequalities globally.[1–4] Vaccination is a crucial measure in breaking the transmission chain of SARS-CoV-2 infections. Among several vaccines against SARS-CoV-2, 13 in clinical development are inactivated vaccines, two of which are already in phase 4 trials. Although the basic cultivation techniques using Vero cells and inactivation strategies are similar, inactivated vaccines differ in the isolated virion strains and the adjuvants used.[5,6] The potential advantages of inactivated vaccines are non-replicability in the host, non-transmissibility, and the induction of a broad range of humoral and cellular

Republic Ministry of Health, İzmir Provincial Health Directorate, İzmir University of Health Sciences Tepecik Training and Research Hospital, İzmir, Turkey (Prof Ş Köse MD); Department of Infectious Diseases, Turkish Republic Ministry of Health, Ankara Provincial Health Directorate, Ankara Training and Research Hospital, Ankara, Turkey (Prof F Ş Erdinç MD); Department of Infectious Diseases and Clinical Microbiology, Bursa Uludağ University Health Application and Research Centre, Bursa Uludağ University Hospital, Bursa, Turkey (Prof E H Akalın MD); Department of Infectious Diseases and Clinical Microbiology, İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine, İstanbul, Turkey (Prof Ö F Tabak MD); Department of Infectious Diseases and Clinical Microbiology, Ege University School of Medicine, İzmir, Turkey (Prof H Pullukçu MD); Department of Chest Diseases, Turkish Republic Ministry of Health, İzmir Provincial Health Directorate, University of Health Sciences Dr Suat Seren Chest Diseases and Surgery Training and Research Hospital, İzmir, Turkey (Ö Batum MD); Department of Infectious Diseases and Clinical Microbiology, İstanbul University, İstanbul School of Medicine, İstanbul, Turkey (Prof S Şimşek Yavuz MD); Department of Infectious Diseases and Clinical Microbiology, Akdeniz University School of Medicine, Antalya, Turkey (Prof Ö Turhan MD); Department of Infectious Diseases and Clinical Microbiology, Turkish Republic Ministry of Health, İstanbul Provincial Health Directorate, Prof Dr Cemil Taşcıoğlu City Hospital, İstanbul, Turkey (M T Yıldırmak MD); Department of Infectious Diseases and Clinical Microbiology, Acıbadem University Atakent Hospital, İstanbul, Turkey (Prof İ Köksal MD); Department of Infectious Diseases and Clinical Microbiology, Çukurova University Balcalı Hospital Health Application and

Research in context

Evidence before this study

We searched PubMed for research articles published up to April 28, 2021, with no language restrictions, using the terms "SARS-CoV-2" OR "COVID-19" AND "vaccine" AND "clinical trial" AND "efficacy". We found four articles reporting the interim efficacy and safety results of phase 3 trials: ChAdOx1 nCoV-19 vaccine (University of Oxford–AstraZeneca) showing an efficacy against symptomatic COVID-19 of 62·1% (95% CI 41·0–75·7) with two standard doses and 90·0% (67·4–97·0) with a low dose followed by a standard dose; Gam-COVID-Vac (Gamaleya National Research Centre for Epidemiology and Microbiology) showing an efficacy of 91·6% (85·6–95·2); mRNA-1273 SARS-CoV-2 vaccine (Moderna) showing an efficacy of 94·1% (89·3–96·8), and BNT162b2 mRNA COVID-19 vaccine (Pfizer–BioNTech) showing an efficacy of 95% (90·3–97·6). The results of the ENSEMBLE trial showed that the efficacy of a single dose of the Ad26.COV2.S vaccine (Janssen Research and Development) against moderate to severe or critical COVID-19 with onset at least 14 days after administration was 66·9% (adjusted 95% CI 59·0–73·4) and at least 28 days after administration was 66·1% (55·0–74·8), and higher efficacies were obtained for severe or critical COVID-19. In the world's first publicly reported animal trial of a SARS-CoV-2 candidate vaccine PiCoVacc, thereafter named CoronaVac in clinical trials, Gao and colleagues showed that the vaccine induced the production of SARS-CoV-2-specific neutralising antibodies in animals and provided complete protection against SARS-CoV-2 challenge in non-human primates. Phase 1/2 studies of CoronaVac showed a good safety and tolerability profile, and a dosage of 3 μg produced seroconversion rates of 92·0% with a 14-day immunisation schedule and 97·0% with a 28-day schedule in participants aged 18–59 years, and 98·0% with a 28-day schedule in participants aged 60 years and older in phase 2 trials.

Added value of this study

This study reports the interim analysis of a double-blind, randomised, placebo-controlled phase 3 clinical trial to assess the efficacy and safety of the inactivated and aluminium hydroxide-adsorbed SARS-CoV-2 vaccine in Turkey, in which both high-risk health-care workers and volunteers with an average COVID-19 exposure risk in the community were recruited. CoronaVac showed an efficacy of 83·5% for preventing PCR-confirmed symptomatic COVID-19, with no cases of COVID-19 requiring hospitalisation. The incidence of adverse events was low (18·9%). Preliminary immunogenicity results revealed that CoronaVac induced anti-receptor-binding domain antibodies in 89·7% of participants. The vaccine is stored and transported at 2–8°C and was granted emergency use authorisation for mass vaccination in Turkey on Jan 13, 2021.

Implications of all the available evidence

The world needs every possible dose of any safe and effective vaccine against SARS-CoV-2. Although novel genetic vaccine production platforms hold great potential for the rapid and adaptable mass production of vaccines, traditional platforms have a long experience of producing safe and tolerable vaccines with good immunogenicity. The results of this interim analysis have shown that CoronaVac fulfils the critical or minimal requirement of vaccines for the indication of pandemic use, hitting above the minimum efficacy of 50% as specified by the WHO target product profile as an option for mass vaccination. WHO has given emergency use approval to another inactivated vaccine from a different Chinese producer (Sinopharm-Beijing) and our results add to the existing evidence on safety and efficacy of inactivated vaccines for prevention of COVID-19.

responses against different epitopes. Their production and scale-up are relatively easy in the context of good yield production systems and the availability of biosafety level 3 facilities.[7] Disadvantages include limited immunogenicity requiring adjuvants to enhance the immune response, large quantities of live virus to be handled, and the integrity of antigens or epitopes that should be verified.[8]

CoronaVac, an inactivated whole-virion SARS-CoV-2 vaccine candidate developed by Sinovac Life Sciences (Beijing, China), has been in phase 3 trials since mid-2020 in Brazil, Indonesia, Chile, and Turkey. As of April 28, 2021, it has been approved in 22 countries for emergency use.[9] In this Article, we present the interim safety and efficacy results of a phase 3 trial in Turkey investigating the use of CoronaVac in adults.

Methods

Study design and participants

We did a double-blind, randomised, placebo-controlled, case-driven phase 3 clinical trial to assess the safety and efficacy of the inactivated SARS-CoV-2 vaccine CoronaVac among volunteers in Turkey.

Volunteers aged 18–59 years with no history of COVID-19 were screened for eligibility. Exclusion criteria included (but were not limited to) positive PCR and total antibody tests for SARS-CoV-2; pregnancy, breastfeeding; known allergy to components of the study vaccine or placebo; recent (within the past 6 months) or planned use of immunosuppressive therapy, or use of immunoglobulins or any blood products within the past 3 months; asplenia; history of bleeding disorder; alcohol or drug abuse; and any confirmed or suspected autoimmune or immunodeficiency disease. The study protocol containing the full list of eligibility criteria is available online.[10]

Participants were recruited in two consecutive cohorts (K1 and K2) at 24 centres (appendix p 8) in Turkey between Sept 15, 2020, and Jan 6, 2021. K1 included actively working health-care workers such as doctors, nurses, and technicians working in health-care facilities,

including but not confined to COVID-19 areas, and was launched to closely observe the safety of the vaccine before proceeding with the community. K2 included subjects representing the community in addition to health-care workers included in K1.

During the study, the Ministry of Health gave an emergency use authorisation for CoronaVac on Jan 13, 2021, and started an immediate vaccination programme initially for health-care workers and later for the public, prioritising older adults (aged ≥65 years). Although recruitment of volunteers was ongoing at this time, to comply with the principles of the Declaration of Helsinki regarding using a placebo for human subjects in medical research, the ethics committee suggested discontinuing the masking and injection of participants in the placebo group. Consequently, the placebo recipients were offered vaccines, first in K1 and later in K2.

The study protocol was approved by the clinical research ethics board of Hacettepe University (approval number 2020/10-26, July 16, 2020). The entire study protocol was published previously and is available on the Hacettepe University Vaccine Institute website.[10] Signed informed consent was obtained from participants before screening.

Randomisation and masking

Randomisation into vaccine and placebo groups was done on day 0, at a 1:1 ratio in K1 and a 2:1 ratio in K2, using an interactive web response system (Omega-CRO, Ankara, Turkey). Participants and practitioners were masked to the group allocation. The masking was removed in the event of a medical emergency requiring acute intervention, upon the responsible investigator's approval and the data and safety monitoring board's knowledge.

Procedures

Oropharyngeal and nasopharyngeal swabs were obtained from all participants for baseline PCR testing with a Bio-Speedy Direct RT-qPCR SARS-CoV-2 detection kit (Bioeksen, Istanbul, Turkey) on a Bio-Rad CFX96 Touch platform (Hercules, CA, USA), and serum total SARS-CoV-2 antibody testing was done. The ADVIA Centaur COV2T assay (Siemens Healthcare Diagnostics, Erlangen, Germany), a fully automated one-step antigen sandwich immunoassay using acridinium ester chemiluminescence technology, was used to detect total antibodies (IgG and IgM) against the SARS-CoV-2 spike protein receptor-binding domain (RBD) in serum samples. This assay is semiquantitative and has a lower detection threshold value (1 sample-to-cutoff ratio). All PCR and serum antibody tests were done at two central laboratories.

The study vaccine is an inactivated whole-virion vaccine with aluminium hydroxide as the adjuvant, prepared with a novel coronavirus (CZ02 strain) inoculated in African green monkey kidney cells (Vero cells). The inactivation process is done by adding β-propiolactone in the virus harvest fluid at a ratio of 1:4000 and inactivating at 2–8°C for 12–24 h. One dose of COVID-19 vaccine contains 3 μg of SARS-CoV-2 virion in a 0·5 mL aqueous suspension for injection with 0·45 mg/mL of aluminium. The placebo contained all ingredients except the inactivated virus, in prefilled syringes. The injections were given in two doses, 14 days apart, intramuscularly in the deltoid muscle. As the placebo and study vaccine looked exactly the same, they were administered by staff masked to group allocation. Details of the procedures on visit dates and the pharmacological properties of the investigational product are provided in the appendix (pp 1–2).

Symptom-based active surveillance was done to detect participants with symptoms suggestive of COVID-19 during follow-up (appendix pp 3–4). Anyone with at least one of the following symptoms for 2 days or more underwent PCR testing: fever or chills; cough; dyspnoea; fatigue; muscle or body pain; headache; new loss of sense of smell or change in taste; sore throat; nasal congestion or rhinorrhoea; nausea or vomiting; and diarrhoea. Cases of SARS-CoV-2 infection were classified according to the scale of clinical progression proposed by WHO.[11] Clinical outcomes were assessed in a blinded manner.

Sampling for immunogenicity analyses was planned in a subgroup of volunteers selected sequentially. As the immunogenicity and T-cell response analyses are ongoing, we only report the initial results of the anti-RBD antibody tests and neutralising antibody assays gathered at least 14 days after the second dose of vaccine or placebo. Virus neutralisation assays were done in an in-house microtitre plate, as described by Hanifehnezhad and colleagues.[12] Five-fold diluted serum samples, starting from 1:5, were mixed with an equal volume of 100 median tissue culture infectious dose of SARS-CoV-2 Ank1 isolate (1:10 000) in quadruplicate and incubated for 1 h at 37°C for neutralisation. The serum–virus mixtures were subsequently inoculated onto 90% confluent Vero E6 cells grown in 96-well plates. The assay was evaluated via inverted microscope when a 100% cytopathic effect was observed in the virus control wells. Reciprocals of serum dilutions inhibiting at least 50% of virus infectivity were expressed as mean antibody titre (SN_{50}).

Outcomes

The primary outcome was the incidence of symptomatic COVID-19 cases confirmed by RT-PCR at least 14 days after the second dose of vaccination, assessed in the per protocol population. Secondary outcomes were the incidence of symptomatic COVID-19 cases confirmed by RT-PCR at least 14 days after the first dose (assessed in all participants who received at least one dose); incidence of hospitalisation or mortality at least 14 days after the second dose; the incidence of COVID-19 cases confirmed by RT-PCR at least 14 days after the second dose; the seroconversion rate, seropositivity rate, geometric mean

Research Centre, Adana, Turkey (Prof Y Taşova MD); **Department of Infectious Diseases and Clinical Microbiology, Marmara University School of Medicine, İstanbul, Turkey** (Prof V Korten MD); **Department of Infectious Diseases and Clinical Microbiology, Karadeniz Technical University School of Medicine, Trabzon, Turkey** (Prof G Yılmaz MD); **Department of Infectious Diseases and Clinical Microbiology, Dicle University School of Medicine, Diyarbakır, Turkey** (Prof M K Çelen MD); **Department of Chest Diseases, Turkish Republic Ministry of Health, İstanbul Provincial Health Directorate, University of Health Sciences İstanbul Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, İstanbul, Turkey** (Prof S Altın MD); **Department of Infectious Diseases and Clinical Microbiology, Turkish Republic Ministry of Health, Kayseri City Training and Research Hospital, Kayseri, Turkey** (Prof İ Çelik MD); **Department of Infectious Diseases and Clinical Microbiology, İnönü University Turgut Özal Health Centre, Malatya, Turkey** (Prof Y Bayındır MD); **Department of Infectious Diseases and Clinical Microbiology, Gaziantep University Şahinbey Research and Application Centre, Gaziantep, Turkey** (Prof İ Karaoğlan MD); **Department of Chest Diseases, Turkish Republic Ministry of Health, Ankara Provincial Health Directorate, Ankara Keçiören Sanatorium, Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital, Ankara, Turkey** (Prof A Yılmaz MD); **Department of Virology, Ankara University Faculty of Veterinary Medicine, Ankara, Turkey** (Prof A Özkul DVM)

Correspondence to:
Prof Murat Akova, Department of Infectious Diseases and Clinical Microbiology, Hacettepe University School of Medicine, Hacettepe Mahallesi, Ankara, 06230, Turkey
makova@hacettepe.edu.tr

See Online for appendix

titre or geometric mean increase in neutralising antibody and IgG 14 days and 28 days after each dose; the incidence of adverse reactions from the day of first vaccination to 28 days after the second dose; the incidence of adverse reactions and adverse events within 7 days after each dose; and the incidence of serious adverse events from the first vaccination to 1 year after the second dose (appendix pp 5–7).

For evaluating the efficacy of CoronaVac, COVID-19-free person-years were calculated for both study groups. Accordingly, the time from the anticipated date of prevention (14 days after the administration of the second dose) to either the date of unmasking or date of an RT-PCR-confirmed diagnosis of COVID-19 was ascertained for each participant and summed to calculate the total person-years without the disease. Total person-years were divided by the number of participants diagnosed with COVID-19 to ascertain the vaccine efficacy in intervention and placebo groups.

Participants were questioned about all adverse events during all visits and through automated phone calls via an interactive voice response system (appendix pp 3–4). Predefined symptoms (solicited events) and other unspecified symptoms (unsolicited events) reported by the participants were recorded. All adverse events were assessed by study investigators for severity and causality. Any adverse event assessed by study investigators as possibly, probably, or definitely related to a study product was defined as an adverse reaction. All safety data, until the date of unmasking and data cutoff, were recorded and analysed in the current report. Further safety data are still being obtained in an open-label follow-up study.

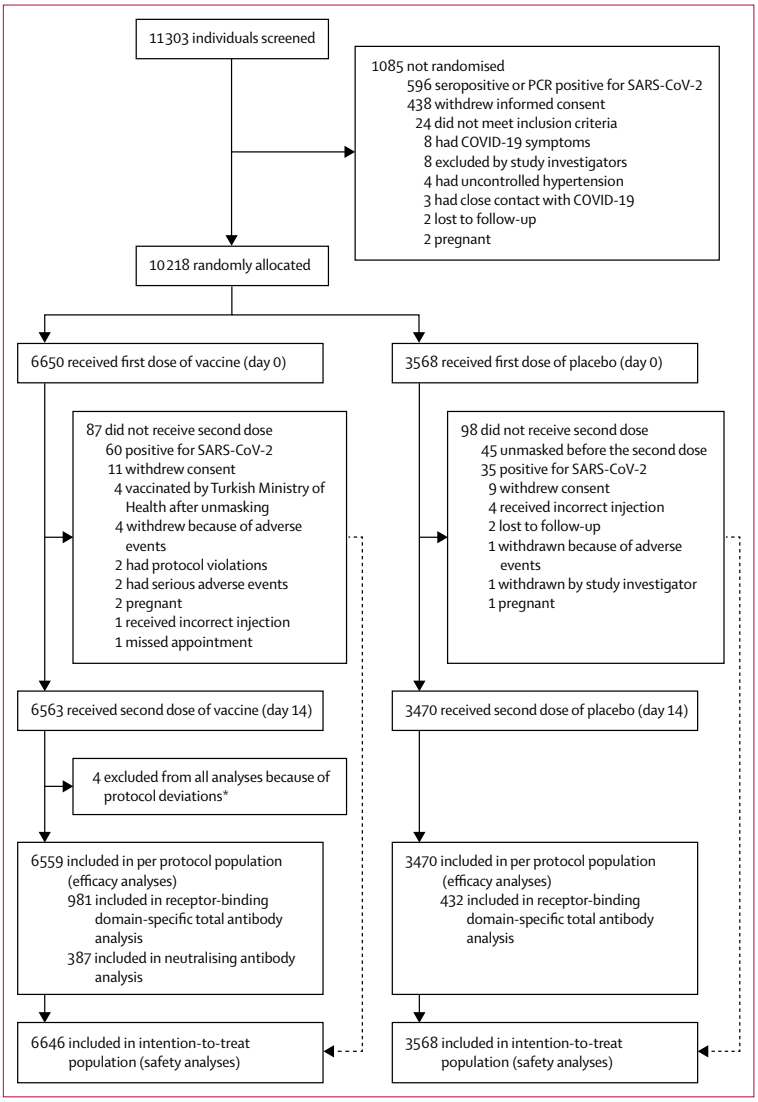


Figure 1: **Trial profile**
*Four participants in the vaccine group received two doses of the study product; however, because they were older than 59 years on the day of randomisation, they were excluded from all safety and efficacy analyses due to protocol violation.

Statistical analysis

For K1, the estimated sample size in both study groups was 588, based on assumptions that the risk of infection with SARS-CoV-2 would be 5% for the placebo group and 2% for the vaccine group. Considering a 10% dropout rate and 5% baseline seropositivity or RT-PCR positivity, it was calculated that 680 subjects would be screened in both groups of K1. Total sample sizes were calculated as 7545 for the vaccine group and 3773 for the placebo group in order to be able to detect a minimum clinically significant difference of 1% (with estimated incidence rates of 1% for the vaccine group and 2% for the placebo group) in a two-sided hypothesis testing design with 95% CIs. With the addition of a 10% dropout rate and 5% seropositivity or RT-PCR positivity at baseline, the total sample size was determined to be 13 000 participants, of whom 1360 would be in K1 and 11 640 in K2.

The initial study protocol indicated that if the efficacy of the vaccine could be demonstrated with an interim analysis done with 40 confirmed cases of COVID-19, masking would be removed and participants in the placebo group would be offered CoronaVac. Because the study was initiated with health-care workers at high risk, it was estimated that 5% of the placebo group (29 participants) and 2% of the vaccine group (11 participants) would have to be infected to demonstrate a clinical efficacy of 60%. If those rates could not be obtained in K1, enrolment would begin for K2. The enrolment rate remained very low for K1 and, after an interim safety analysis on Nov 18, 2020, the data and safety monitoring board decided to start enrolment into K2. Although the prespecified number of COVID-19 cases for the interim efficacy analysis was 40, as the incidence throughout Turkey increased rapidly, the Ministry of Health asked for a preliminary analysis to be able to grant an emergency use authorisation for

CoronaVac. Therefore, a non-predefined interim analysis was done on Dec 24, 2020, with 29 cases, which showed an efficacy above 60%. Afterwards, as community vaccination commenced, study participants were unmasked starting with K1 in blocks. The masked follow-up of those participants continued until their code was unmasked, and 41 COVID-19 cases were attained by the time all of the codes were unmasked and the prespecified interim analyses for efficacy and safety were done. Therefore, the cutoff date for inclusion in the analyses of the primary efficacy outcome and the secondary efficacy outcomes was the unmasking date of each participant in both groups. The follow-up period was defined as the period (days) from the randomisation date to the unmasking date. The data lock date was March 16, 2021. Safety data in the CoronaVac intention-to-treat group were gathered in an unmasked manner after the unmasking date, and an extended safety analysis until the data lock date is also presented.

All analyses were done using SPSS for Windows (version 25.0). Descriptive analyses were presented using mean and SD for continuous variables and frequency and percentage for categorical variables. 95% CI was presented for efficacy, calculated as events per COVID-19-free person-years (ie, the sum of RT-PCR-confirmed COVID-19 cases divided by the sum of time from vaccine protection to diagnosis or unmasking).

Time to diagnosis of COVID-19 from the time of anticipated vaccine protection in both groups was presented with Kaplan-Meier survival curves. Safety analyses were done in the intention-to-treat population. Because the study product is an inactivated vaccine, a single dose was not expected to be as efficacious as two doses, and the primary efficacy analysis was therefore done in the per protocol population (defined as participants who received two doses of vaccine or placebo in accordance with group allocation. To compare adverse events between the study groups, the χ^2 test was used when the χ^2 condition was met; otherwise, Fisher's exact test was used. A Mantel-Haenszel test of trend was used in the analysis of the positive anti-RBD antibody results among age groups within both sexes. A log-rank test was used for the comparison of follow-up duration between the treatment groups. The independent data and safety monitoring board monitored the quality of evidence, adverse events, revisions in line with the current literature, individual privacy, and data reliability from the planning stage to the end of the study.

This study is registered with ClinicalTrials.gov (NCT04582344).

Role of the funding source

The Turkish Health Institutes Association (TUSEB) provided the funding for this study; approved the final protocol, final manuscript, and the decision to submit for publication, but had no role in data collection, data analysis, data interpretation, or writing of the report. Omega-CRO (Ankara, Turkey) acted as the contract research organisation representing TUSEB and contributed to correspondence between investigators, the ethics committee, and the Ministry of Health; monitoring, site management, storage, and distribution of the consumables; developing electronic case report forms, the interactive web response system, and the interactive voice response system; and data management, statistical analyses, and overall project management. Sinovac Life Sciences provided the investigational products and reviewed the data and final manuscript before submission; however, the authors retained editorial control.

Results

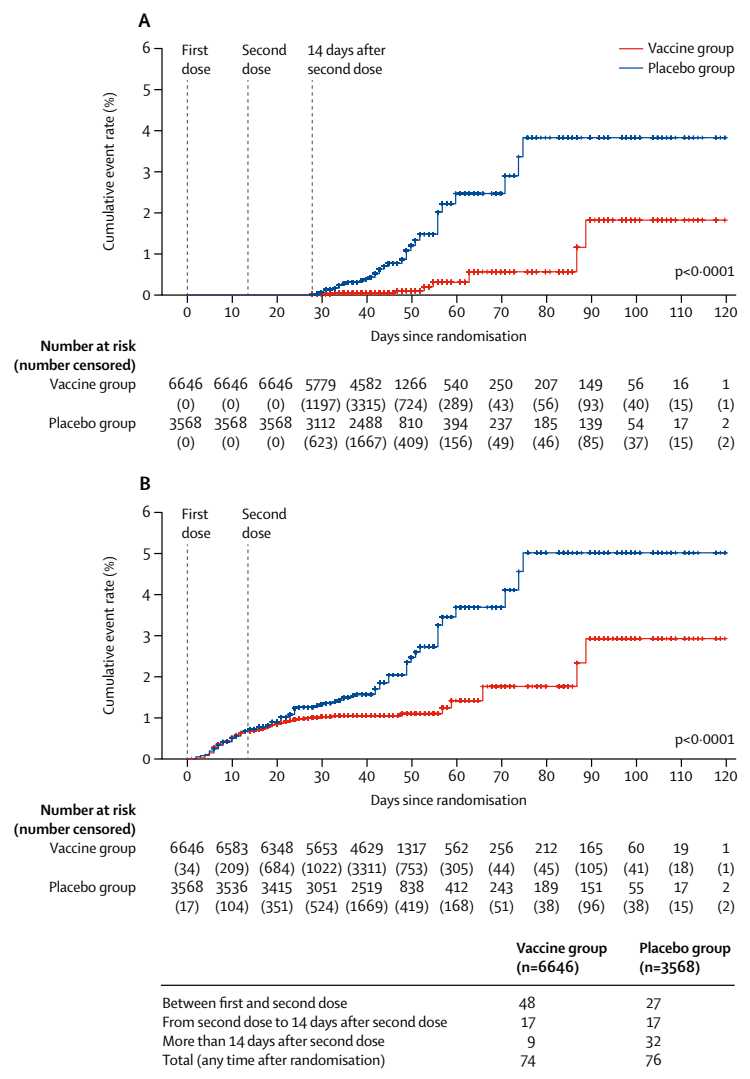
11 303 volunteers were screened for eligibility, and 10 218 were randomly allocated (6650 [65·1%] to the vaccine group and 3568 [34·9%] to the placebo group) between Sept 15, 2020, and Jan 6, 2021 (figure 1). After administration of the first dose and before receiving the second dose, 87 participants in the study group and 98 in the placebo group were excluded. After receiving two doses,

	Vaccine group (n=6646)	Placebo group (n=3568)
Age, years		
Median (IQR)	45 (37–51)	45 (37–51)
18–44	3259 (49·0%)	1764 (49·4%)
45–59	3387 (51·0%)	1804 (50·6%)
Sex		
Female	2831 (42·6%)	1476 (41·4%)
Male	3815 (57·4%)	2092 (58·6%)
Body-mass index*, kg/m²		
Median (IQR)	25·7 (23·2–28·4)	25·7 (23·2–28·4)
<25	2592 (42·5%)	1372 (41·9%)
25–30	2536 (41·6%)	1414 (43·1%)
≥30	971 (15·9%)	492 (15·0%)
Study cohort†		
K1	458 (6·9%)	461 (12·9%)
K2	6188 (93·1%)	3107 (87·1%)
Health-care worker	2297 (34·6%)	1378 (38·6%)
Comorbidities present‡		
Hypertension	483 (11·8%)	249 (11·6%)
Cardiovascular disease other than hypertension	104 (2·6%)	46 (2·1%)
Chronic respiratory disease	118 (2·9%)	63 (2·9%)
Diabetes	199 (4·9%)	97 (4·5%)
Malignancy	36 (0·9%)	14 (0·7%)
Autoimmune or autoinflammatory disease	34 (0·8%)	23 (1·1%)

Data are median (IQR) or n (%). *Data were available for 6099 participants in the vaccine group and 3278 in the placebo group. †919 health-care workers were enrolled into the K1 cohort (1:1 vaccine-to-placebo randomisation ratio), of whom 667 were enrolled before Nov 18, 2020, at which point an interim safety analysis without unmasking revealed that the vaccine had a good safety profile and K2 was initiated; 252 volunteers were further recruited into K1 until Jan 4, 2021, after which the enrolment was solely into K2 (2:1 vaccine-to-placebo randomisation ratio). ‡Data were available for 4076 participants in the vaccine group and 2141 in the placebo group; participants with a medical history of malignancy or autoimmune or autoinflammatory disease did not have active disease at the time of enrolment and were not on immunosuppressive treatment.

Table: **Characteristics of study participants**

four (0·1%) participants in the vaccine group were excluded from all analyses because of protocol deviations (being older than 59 years on the day of randomisation). Finally, 10 214 participants (6646 [65·1%] assigned to the vaccine group and 3568 [34·9%] assigned to the placebo group) formed the intention-to-treat population, and 10 029 participants who received two doses of CoronaVac (6559 [65·4%] participants) or placebo (3470 [34·6%] participants) formed the per protocol population. On the date of data cutoff, 10 214 participants in the intention-to-treat population had reached a median 90 days (IQR 82–102) of follow-up after the first dose. All recruitment, randomisation, and follow-up procedures were completed in 24 study centres (appendix p 8).



	Vaccine group (n=6646)	Placebo group (n=3568)
Between first and second dose	48	27
From second dose to 14 days after second dose	17	17
More than 14 days after second dose	9	32
Total (any time after randomisation)	74	76

Figure 2: **Cumulative incidence curves for COVID-19 cases**
(A) Cumulative incidence of COVID-19 in the per protocol population (assessed by analysing cases occurring 14 days or more after the second dose of vaccination). (B) Cumulative incidence of COVID-19 in the intention-to-treat population (starting immediately after randomisation).

The main characteristics of the participants are shown in the table. The median age of the participants was 45 years (IQR 37–51), and 5191 (50·8%) were older than 45 years. 5907 (57·8%) participants were male, 4307 (42·2%) were female, 3675 (36·0%) were health-care workers, and 1463 (15·6%) were obese (body mass index ≥30 kg/m²). Among 6217 participants with comorbidity data reported, hypertension was the most prevalent condition (732 [11·8%] participants).

150 cases of COVID-19 were observed among 10 214 participants from the date of randomisation to the date of unmasking (median follow-up 43 days [IQR 36-48], incidence rate 122·5 cases [95% CI 104·7–142·2] per 1000 person-years). In the per protocol population (n=10 029), 41 cases of symptomatic COVID-19 occurred at least 14 days after the second dose of vaccine or placebo (91·1 cases [66·2–121·6] per 1000 person-years). Of these cases, nine were reported in the vaccine group (n=6559; 31·7 cases [14·6–59·3] per 1000 person-years) and 32 in the placebo group (n=3470; 192·3 cases [135·7–261·1] per 1000 person-years), yielding a vaccine efficacy of 83·5% (95% CI 65·4–92·1; p<0·0001) for the prevention of PCR-confirmed symptomatic COVID-19.

Cumulative incidences of COVID-19-related events in the vaccine and placebo groups are shown in figure 2. There were no fatal cases of COVID-19. Hospitalisation was recorded in none of the participants in the vaccine group and six in the placebo group (36·4 hospitalisations [13·5–77·5] per 1000 person-years), giving a vaccine efficacy of 100% (20·4–100·0; p=0·0344) for the prevention of COVID-19-related hospitalisation. The distribution of COVID-19 cases with regard to the WHO Clinical Progression Scale is given in the appendix (p 9). 20 PCR-confirmed symptomatic COVID-19 cases occurred between days 14 and 27 after the first dose in both groups (efficacy 46·4% [0·4–71·2], p=0·0486).

1413 participants (981 in the vaccine group and 432 in the placebo group) were involved in the immunogenicity analyses. 880 (89·7%) vaccine recipients and 19 (4·4%) placebo recipients were seropositive for RBD-specific total antibody (p<0·0001; figure 3). Seropositivity decreased with increasing age in women (p_{trend}=0·0003) and men (p_{trend}=0·0084). Virus neutralisation assays in selected samples (n=387) from seropositive participants in the vaccine group showed SN_{50}s of at least 1/15 in 356 (92·0%) of the tested samples (figure 4).

Analyses of adverse events were done in the intention-to-treat population, which excluded four participants who had protocol deviations (n=10 214; figure 1). The vaccine showed a satisfactory safety profile, with no grade 4 adverse events or deaths during the study period. Six (0·1%) of 6646 participants in the vaccine group and one (<0·1%) of 3568 in the placebo group were withdrawn from the study because of adverse events. 3845 adverse events were

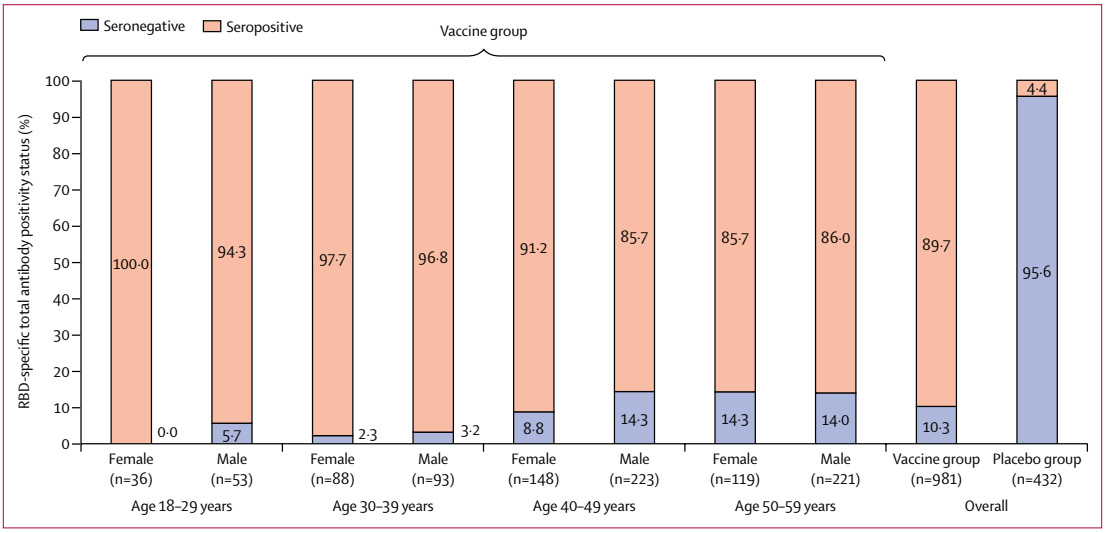


Figure 3: **Seropositivity of RBD-specific total antibodies in the vaccine and placebo groups 14 days after the second dose, by age and sex**
The participants with positive RBD-specific antibodies in the placebo group neither reported any symptoms during the follow-up nor had a laboratory confirmed diagnosis of COVID-19, probably representing cases with asymptomatic SARS-CoV-2 infection. RBD=receptor-binding domain.

reported among 1862 participants (1259 [18·9%] in the vaccine group and 603 [16·9%] in the placebo group, p=0·0108; figure 5A). Adverse events resolved in a median of 1 day (IQR 0–2). 3242 (84·3%) of 3845 adverse events were solicited (predefined) events, and were higher in the vaccine group (1148 [17·3%] participants) than in the placebo group (537 [15·1%], p=0·0039). Unsolicited (non-predefined) adverse events had a low incidence in both groups (figure 5A). Among all adverse events, 3469 (90·2%) were grade 1 and 3365 (87·5%) occurred within 7 days after injection. A comprehensive breakdown of adverse events is provided in the appendix (pp 10–14).

Local reactions were more commonly reported in vaccine recipients (180 [2·7%] participants) than in placebo recipients (52 [1·5%], p<0·0001). The most common solicited local reaction was inoculation site pain, which occurred significantly more frequently in the vaccine group (157 [2·4%] participants) than in the placebo group (40 [1·1%], p<0·0001). Other local adverse events, including erythema, paraesthesia, and swelling, were rare and did not differ significantly in incidence between groups (figure 5B).

The frequency of systemic adverse events was significantly higher in the vaccine group (1179 [17·7%] participants) than in the placebo group (571 [16·0%], p=0·0263). Events reported more frequently in the vaccine group than in the placebo group included fatigue (546 [8·2%] in the vaccine group *vs* 248 [7·0%] in the placebo group, p=0·0228), myalgia (267 [4·0%] *vs* 106 [3·0%], p=0·0071), chill (164 [2·5%] *vs* 63 [1·8%], p=0·0217), and nausea (46 [0·7%] *vs* 7 [0·2%], p=0·0008; figure 5C).

11 (0·1%) participants had serious adverse events during the study period (six [0·1%] in the vaccine group

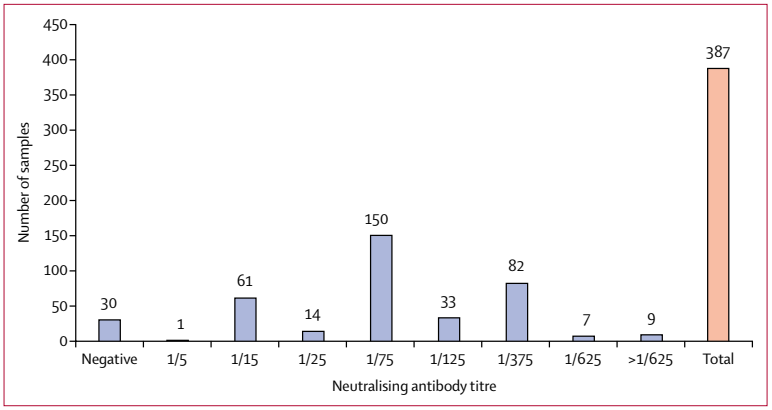


Figure 4: **Neutralising antibody titres among the subset of participants included in the immunogenicity analysis**

and five [0·1%] in the placebo group; appendix pp 10–14). Initially, two serious adverse events in the vaccine group were reported to have a causal relationship with the vaccine. The first participant had a grade 3 systemic allergic reaction that occurred more than 24 h after the administration of the first dose of vaccine and resolved uneventfully in the following 24 h. The other participant presented with seizure 43 days after the second dose of the vaccine; however, after an extensive work-up, this patient was diagnosed with an infiltrative glial neoplasm and, in the final assessment, this adverse event was judged to be unrelated to the vaccine.

Discussion

This interim analysis indicated that, in a population aged 18–59 years, CoronaVac had high efficacy for preventing

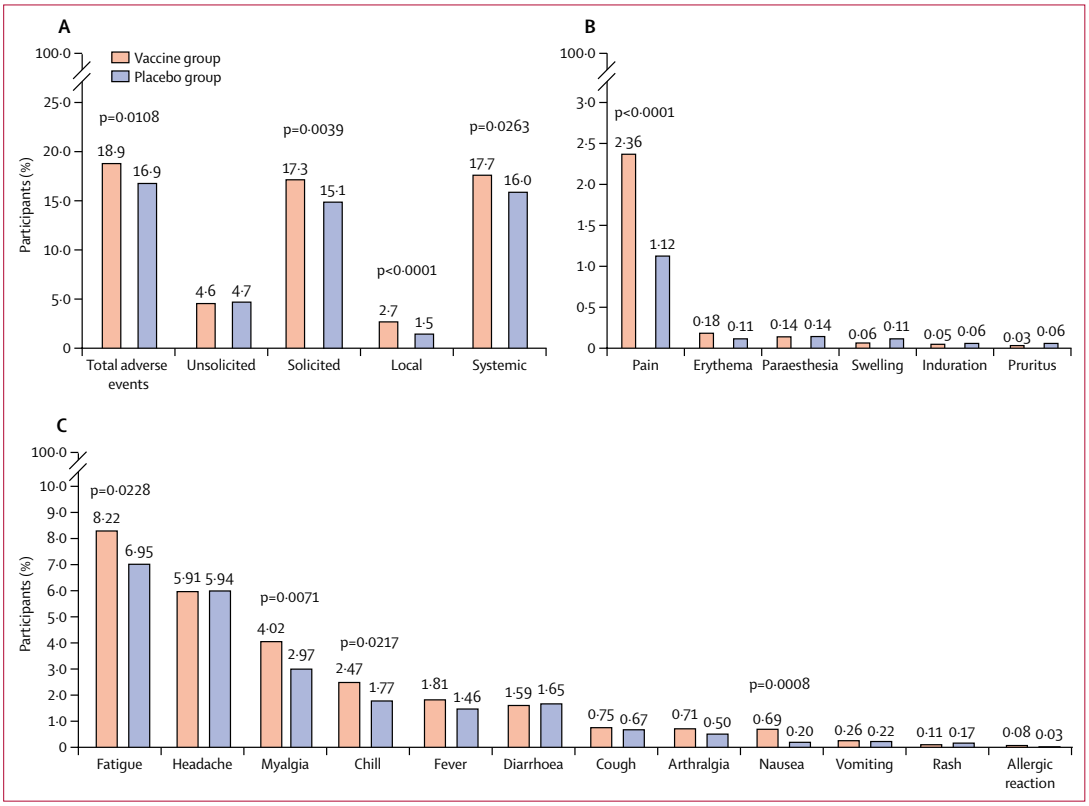


Figure 5: **Adverse events**
(A) Overall adverse events. (B) Local adverse events. (C) Systemic adverse events. p values are shown only for significant differences. See appendix (pp 10–12) for full data.

symptomatic COVID-19 (83·5% relative to placebo) and COVID-19-related hospitalisation (100%) at least 14 days after the second dose. Efficacy in subgroups was not a secondary outcome and the trial was not designed or powered to analyse the efficacy of the vaccine with regard to demographic variables and risk factors. Such analyses will require further trials designed accordingly. Anti-RBD antibodies developed in 89·7% of volunteers in a subset of our study sample, and 92·0% of those who were seropositive also produced protective levels of neutralising antibodies at least 14 days after the second dose of vaccine.

Inactivated SARS-CoV-2 vaccine candidates have shown promising results in preclinical trials.[13-15] Gao and colleagues[13] showed that, in mice, rats, and rhesus monkeys, 6 μg CoronaVac induced SARS-CoV-2-specific neutralising antibodies that effectively neutralised ten representative SARS-CoV-2 strains and provided complete protection against SARS-CoV-2 challenge in non-human primates. BBV152 (manufactured by Bharat Biotech), another inactivated vaccine, generated a quick and robust immune response with no histopathological changes in the lungs upon SARS-CoV-2 challenge in animal studies, provided adequate protection against SARS-CoV-2 infection in rhesus monkeys, induced T-helper-1 cell-skewed immune responses with elevated IgG2a/IgG1 ratios, and increased levels of SARS-CoV-2-specific IFNγ$^+$CD4$^+$ T-lymphocyte responses.[15,16] A phase 1 trial also revealed moderate seroconversion rates that persisted for up to 3 months after the second dose.[17,18] The immune response elucidated with inactivated vaccines is not confined just to the spike protein but rather to other SARS-CoV-2 proteins—the matrix proteins, envelope proteins, and nucleoprotein—which theoretically could be reflected as a vast array of immunogenic responses.[6,7] Voss and colleagues[19] showed that, in people previously infected with SARS-CoV-2, the plasma IgG response against SARS-CoV-2 was oligoclonal and more than 80% of spike protein IgG antibodies were directed towards non-RBD epitopes in the spike protein. This finding indicates that non-RBD-directed antibodies might have a role in protection against SARS-CoV-2 infection.

Phase 1/2 trials of CoronaVac in volunteers aged 18–59 years and older than 60 years showed that the vaccine doses and schedules investigated (3 μg or 6 μg, applied 14 days or 28 days apart) all had similar safety and immunogenicity profiles.[20,21] Considering the production

capacity and emergent need for vaccines, the 3 µg dose of CoronaVac has been suggested for efficacy assessment.[20] Palacios and colleagues[22] reported an overall efficacy of CoronaVac against symptomatic COVID-19 of 50·7% (95% CI 36·0–62·0) 14 days or more after the second dose; however, the efficacy in preventing the need for assistance (defined as a score ≥3 on the WHO Clinical Progression Scale) was 83·7% (58·0–93·7) and efficacy against moderate and severe cases was 100% (56·4–100·0). In a subset of participants, neutralising antibody assays showed that there were no significant differences in the frequency of seroconversion or geometric mean titres of neutralising antibodies against the B.1.128 variant compared with those against the P.1 and P.2 variants. The study cohort only included health-care workers actively working with COVID-19 patients, and a PCR-positive case with local symptoms (such as sore throat, nasal congestion, or rhinorrhoea) was considered as a failure of the vaccine, thus indicating that the vaccine might confer lower protection against asymptomatic or mildly symptomatic cases. The interim report of the phase 3 trial in Chile with a subset of 434 health-care workers, including those aged 60 years or older, revealed high seroconversion rates for specific anti-S1-RBD IgG and neutralising antibodies, along with a robust T-cell response.[23] The interim phase 3 results of other COVID-19 vaccines have shown efficacies ranging from 62·1% to 95%.[24–28] Higher and more rapidly established efficacies were observed with mRNA-based vaccines.[25,26] Considering the immunogenic mechanisms of inactivated vaccines, because one dose is not expected to be as efficacious as two doses, we did not expect to and could not show an early protective effect after the first dose, in contrast to findings with mRNA vaccines.

The tolerability of CoronaVac in this study was excellent and the incidence of adverse events, most of which were solicited systemic events, was low. The majority of the adverse events were grade 1 and occurred within 7 days after the injection. No grade 4 adverse events were observed and there was only one adverse event (an allergic reaction) that required hospitalisation.

The targeted sample size could not be reached because CoronaVac was granted emergency use authorisation by the Turkish Ministry of Health while the study recruitment was ongoing, and an immediate vaccination programme was initiated for health-care workers and later for the general public in Turkey. To comply with ethical standards, recruitment was closed earlier than planned and the placebo recipients were offered vaccines, depending on their vaccination priority.

The strengths of this study include the low dropout rate, reflecting the good tolerability of the vaccine. Additionally, the participants were from different risk groups and occupations, rendering the results of the study more generalisable to the real-world context. Additionally, active symptom surveillance was pursued to detect COVID-19 cases.

This study also has several limitations. First, the median follow-up period after randomisation to the date of unmasking was 43 days (IQR 36–48), which is a very short duration of follow-up. It is not possible to comment on the long-term protective effects of the two-dose immunisation schedule with this interim analysis.

Second, one should bear in mind that the study population consisted of relatively young (median age 45 years [37–51]) and healthy individuals with a low prevalence of chronic diseases, and the overall event rate was very low. Therefore, the generalisability of the findings of this interim analysis needs to be evaluated cautiously. In particular, the number of patients hospitalised with COVID-19 was quite low and the study population consisted of individuals at relatively low risk of severe or critical COVID-19, restricting our ability to make generalised conclusions about severe disease.

Third, the study used a 14-day interval immunisation scheme, whereas the community immunisation was with a 28-day interval. It has been claimed that, although 28-day immunisation schemes elucidated better immunogenicity after the second dose, longer intervals between the two doses are correlated with a higher probability of contracting COVID-19 before getting fully immunised and a great chance of emergence of mutant variants that can replicate in the setting of suboptimal levels of neutralising antibodies.[29] As our results pertain to the data before the emergence of variants of concern, we cannot comment on the efficacy of CoronaVac on the prevention of infection with mutant viruses. Although one of the prespecified outcomes was seroconversion, we have avoided using this term in our reporting of the results because the immunoassay we used was a semiquantitative assay. In fact, all of the participants were seronegative at the time of screening; therefore, the seropositivity 14 days after the second dose of vaccine would indicate seroconversion. However, we could not exclude the possibility that some samples with antibody levels below a sample-to-cutoff ratio of 1 might have very low concentrations of established antibodies. The current report neither involves data on the sequential serum neutralising antibody titres nor the magnitude of T-cell responses or the duration of protectivity. However, a study setting has been established to analyse the proliferation and functional capacity of $CD4^+$ and $CD8^+$ T cells, and the results of an initial study in a group of COVID-19 survivors have been reported by Tavukcuoglu and colleagues.[30] This setting is now being used to analyse the samples from selected participants of this trial to show the functional capacity of T cells induced by CoronaVac to reinvigorate antiviral immunity against SARS-CoV-2.

In summary, our results show that CoronaVac has good efficacy against symptomatic SARS-CoV-2 infection and severe COVID-19 (ie, that requiring hospitalisation), along with a very good safety profile in a population aged 18–59 years. Because this analysis included a very short

follow-up period before the emergence of viral variants and included a young and low-risk population, further data are needed on the performance of CoronaVac to demonstrate the efficacy of the vaccine against the variants of concern and the duration of protection, and to assess the safety and efficacy in older adult populations, adolescents, and children, and individuals with specific chronic diseases.

Contributors

The principal investigators, SU and MA, conceptualised and coordinated the study. SU, MA, MDT, and HLD drafted the manuscript. SU, MA, MDT, and HLD accessed and verified the data and contributed to the analysis and interpretation of the data. SU, MA, MDT, and HLD edited the manuscript. All authors were involved in organisation, coordination, conduct, and technical support of the study; collected data; critically reviewed the manuscript and approved the final version; had full access to all data in the studies, and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

Anonymous participant data will be available upon completion of the clinical trial and publication of the completed study results upon request to the corresponding author. Proposals will be reviewed and approved by the sponsor, researchers, and staff, on the basis of scientific merit and absence of competing interests. Once the proposal has been approved, data can be transferred through a secure online platform after signing a data access agreement and a confidentiality agreement.

Acknowledgments

We are grateful to all participants who volunteered to be part of this study and to all members of the clinical research teams of the participating sites. We thank TUSEB for funding the study and Omega-CRO for the statistical analyses and production of figures, and providing the study protocol. We also thank the members of the data and safety monitoring board for their contributions in the safe execution of this study.

References

1 Brooks SK, Webster RK, Smith LE, et al. The psychological impact of quarantine and how to reduce it: rapid review of the evidence. *Lancet* 2020; **395:** 912–20.

2 Kniffin KM, Narayanan J, Anseel F, et al. COVID-19 and the workplace: implications, issues, and insights for future research and action. *Am Psychol* 2021; **76:** 63–77.

3 WHO. Health inequity and the effects of COVID-19: assessing, responding to and mitigating the socioeconomic impact on health to build a better future. 2020. https://apps.who.int/iris/handle/10665/338199 (accessed June 30, 2021).

4 Dorn AV, Cooney RE, Sabin ML. COVID-19 exacerbating inequalities in the US. *Lancet* 2020; **395:** 1243–44.

5 Yan ZP, Yang M, Lai CL. COVID-19 vaccines: a review of the safety and efficacy of current clinical trials. *Pharmaceuticals (Basel)* 2021; **14:** 406.

6 Iversen PL, Bavari S. Inactivated COVID-19 vaccines to make a global impact. *Lancet Infect Dis* 2021; **21:** 746–48.

7 Krammer F. SARS-CoV-2 vaccines in development. *Nature* 2020; **586:** 516–27.

8 Awadasseid A, Wu Y, Tanaka Y, Zhang W. Current advances in the development of SARS-CoV-2 vaccines. *Int J Biol Sci* 2021; **17:** 8–19.

9 McGill COVID19 Vaccine Tracker Team. Sinovac: CoronaVac. https://covid19.trackvaccines.org/vaccines/7/ (accessed April 28, 2021).

10 Akova M, Unal S. A randomized, double-blind, placebo-controlled phase III clinical trial to evaluate the efficacy and safety of SARS-CoV-2 vaccine (inactivated, Vero cell): a structured summary of a study protocol for a randomised controlled trial. *Trials* 2021; **22:** 276.

11 WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis* 2020; **20:** e192–97.

12 Hanifehnezhad A, Kehribar EŞ, Öztop S, et al. Characterization of local SARS-CoV-2 isolates and pathogenicity in IFNAR$^{-/-}$ mice. *Heliyon* 2020; **6:** e05116.

13 Gao Q, Bao L, Mao H, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. *Science* 2020; **369:** 77–81.

14 Wang H, Zhang Y, Huang B, et al. Development of an inactivated vaccine candidate, BBIBP-CorV, with potent protection against SARS-CoV-2. *Cell* 2020; **182:** 713–21.e9.

15 Mohandas S, Yadav PD, Shete-Aich A, et al. Immunogenicity and protective efficacy of BBV152, whole virion inactivated SARS-CoV-2 vaccine candidates in the Syrian hamster model. *iScience* 2021; **24:** 102054.

16 Ganneru B, Jogdand H, Daram VK, et al. Th1 skewed immune response of whole virion inactivated SARS CoV 2 vaccine and its safety evaluation. *iScience* 2021; **24:** 102298.

17 Ella R, Vadrevu KM, Jogdand H, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: a double-blind, randomised, phase 1 trial. *Lancet Infect Dis* 2021; **21:** 637–46.

18 Ella R, Reddy S, Jogdand H, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: interim results from a double-blind, randomised, multicentre, phase 2 trial, and 3-month follow-up of a double-blind, randomised phase 1 trial. *Lancet Infect Dis* 2021; published online March 8. https://doi.org/10.1016/s1473-3099(21)00070-0.

19 Voss WN, Hou YJ, Johnson NV, et al. Prevalent, protective, and convergent IgG recognition of SARS-CoV-2 non-RBD spike epitopes. *Science* 2021; **372:** 1108–12.

20 Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21:** 181–92.

21 Wu Z, Hu Y, Xu M, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21:** 803–12.

22 Palacios R, Batista AP, Albuquerque CSN, et al. Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in Brazil: the PROFISCOV Study. *SSRN* 2021; published online April 14. https://ssrn.com/abstract=3822780 (preprint).

23 Bueno SM, Abarca K, González PA, et al. Safety and immunogenicity of an inactivated vaccine against SARS-CoV-2 in healthy Chilean adults in a phase 3 clinical trial. *medRxiv* 2021; published online April 1. https://doi.org/10.1101/2021.03.31.21254494 (preprint).

24 Logunov DY, Dolzhikova IV, Shcheblyakov DV, et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. *Lancet* 2021; **397:** 671–81.

25 Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* 2021; **384:** 403–16.

26 Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N Engl J Med* 2020; **383:** 2603–15.

27 Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021; **397:** 99–111.

28 Sadoff J, Gray G, Vandebosch A, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against COVID-19. *N Engl J Med* 2021; **384:** 2187–201.

29 Saad-Roy CM, Morris SE, Metcalf CJE, et al. Epidemiological and evolutionary considerations of SARS-CoV-2 vaccine dosing regimes. *Science* 2021; **372:** 363–70.

30 Tavukcuoglu E, Horzum U, Cagkan Inkaya A, Unal S, Esendagli G. Functional responsiveness of memory T cells from COVID-19 patients. *Cell Immunol* 2021; **365:** 104363.

1.17. Eficácia global da CoronaVac pode chegar a 62,3% com intervalo entre doses igual ou superior a 21 dias

Um artigo científico encaminhado por cientistas do Butantan para a revista científica The Lancet mostrou que a eficácia da CoronaVac para casos sintomáticos atingiu 50,7% com 14 dias de intervalo entre as duas doses, mais do que os 50,38% divulgados em janeiro com base nos dados iniciais do estudo clínico de fase 3. Além disso, a eficácia global, que aponta a capacidade que o imunizante tem de proteger em casos leves, moderados ou graves, pode chegar a 62,3% se o espaço entre as duas doses for de 21 dias ou mais.

Os dados fazem parte de um aprofundamento dos estudos clínicos realizados em 2020 com mais de 12 mil participantes, todos profissionais da saúde. A pesquisa foi liderada pelo diretor de ensaios clínicos do Instituto Butantan, Ricardo Palacios. O artigo ainda diz que a eficácia mínima da vacina já aparece na segunda semana depois da primeira dose. Porém, para que a imunização fique completa, é necessário receber as duas doses.

Inicialmente, o estudo clínico de fase 3 indicava que, para os casos moderados e graves, que necessitam de assistência médica, a eficácia da vacina variava entre 78% e 100%. Nos resultados da nova pesquisa, no entanto, o imunizante se mostrou eficaz entre 83,7% e 100% dos casos. Isso significa que a CoronaVac tem a capacidade de reduzir a maioria dos casos que exige algum cuidado médico.

O artigo ainda sugere que a CoronaVac, imunizante desenvolvido em parceria com a biofarmacêutica chinesa Sinovac, é capaz de proteger contra as variantes P.1 e P.2 do novo coronavírus.

O estudo foi conduzido entre 21 de julho e 16 de dezembro de 2020. Foram 12.396 voluntários em 16 centros de pesquisa brasileiros, e todos receberam ao menos uma dose da vacina ou placebo. No total, 9.823 participantes receberam as duas doses. Não houve óbitos por Covid-19 durante os testes.

Os dados foram divulgados na plataforma de preprints da revista The Lancet.

Publicado em: 14/04/2021

Article

Title Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in Brazil: The PROFISCOV study

Ricardo Palacios, MD, PhD [1*]
Ana Paula Batista, RN [1]
Camila Santos Nascimento Albuquerque, RN [1]
Elizabeth González Patiño, PhD [1]
Joane do Prado Santos, BTech [1]
Mônica Tilli Reis Pessoa Conde, MD, PhD [1]
Roberta de Oliveira Piorelli, MD [1]
Luiz Carlos Pereira Júnior, MD, PhD [2]
Sonia Mara Raboni, MD, PhD [3]
Fabiano Ramos, MD, PhD [4]
Gustavo Adolfo Sierra Romero, MD, PhD [5]
Fábio Eudes Leal, MD, PhD [6]
Luis Fernando Aranha Camargo, MD, PhD [7]
Francisco Hideo Aoki, MD, PhD [8]
Eduardo Barbosa Coelho, MD, PhD [9]
Danise Senna Oliveira, MD, PhD [10]
Cor Jesus Fernandes Fontes, MD, PhD [11]
Gecilmara Cristina Salviato Pileggi, MD, PhD [12]
Ana Lúcia Lyrio de Oliveira, MD, PhD [13]
André Machado de Siqueira, MD, PhD [14]
Danielle Bruna Leal de Oliveira, PhD [15]

Viviane Fongaro Botosso, PhD [16]

Gang Zeng, PhD [17]

Qianqian Xin, PhD [17]

Mauro Martins Teixeira, MD, PhD [18]

Maurício Lacerda Nogueira, MD, PhD [19]

Esper G Kallas, MD, PhD [20]

On behalf of the PROFISCOV study group.

1. Clinical Trials and Pharmacovigilance Center, Instituto Butantan, São Paulo, Brazil.

2. Emilio Ribas Institute of Infectious Diseases. Secretary of Health of Sao Paulo State, São Paulo, SP, Brazil

3. Department of Infectious Diseases, Hospital de Clínicas, Universidade Federal do Paraná, Curitiba, PR, Brazil

4. Infectious Diseases Service, Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil.

5. Center for Tropical Medicine, School of Medicine, University of Brasilia, Brasília, DF, Brazil

6. Universidade Municipal de São Caetano do Sul, São Caetano do Sul, SP, Brazil

7. Instituto Israelita de Ensino e Pesquisa Albert Einstein, São Paulo, SP, Brazil

8. School of Medical Science and Hospital of Clinics, State University of Campinas – UNICAMP, Campinas, SP, Brazil

9. Department of Internal Medicine, Ribeirão Preto Medical School, University of Sao Paulo, Ribeirão Preto, SP, Brazil.

10. Internal Medicine Department. School of Medicine, Universidade Federal de Pelotas. Pelotas, RS, Brazil

11. Department of Internal Medicine and Infectious Diseases, Julio Müller School Hospital, Federal University of Mato Grosso, Cuiaba, MT, Brazil.

12. Research Institute of Cancer Hospital, Barretos, SP, Brazil

13. Hospital Universitário Maria Aparecida Pedrossian, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil

14. Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

15. Laboratory of Clinical and Molecular Virology, Department of Microbiology, Institute of Biomedical Science, University of Sao Paulo, São Paulo, SP, Brazil

16. Virology Laboratory, Development and Innovation Center (CDI) Instituto Butantan, Sao Paulo, SP, Brazil

17. Sinovac Biotech Co., Ltd, Haidian District, Beijing, People's Republic of China

18. Centro de Pesquisa e Desenvolvimento de Fármacos, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

19. Faculdade de Medicina de São José do Rio Preto (FAMERP), São José de Rio Preto, SP, Brazil

20. Department of Infectious and Parasitic Diseases, Clinicas Hospital, School of Medicine, University of São Paulo, São Paulo, SP, Brazil.

* Corresponding author: Ricardo Palacios, Clinical Research Medical Director, Instituto Butantan, São Paulo, Brazil, ricardo.palacios@butantan.gov.br, Phone +55-11-3723-2121

Abstract

Background

Vaccines are urgently needed to tackle the unprecedented morbidity and mortality of COVID-19. Administration of inactivated viruses are the common and mature platform of developing new vaccines. CoronaVac is an inactivated vaccine that has undergone preclinical tests and phase I/II clinical trials.

Methods

We conducted a randomised, double-blind, placebo-controlled phase 3 clinical trial with CoronaVac among healthy healthcare professionals in 16 centres in Brazil. Participants received two doses of vaccine (3 μg in 0.5 mL) vaccine or placebo at day 0 and 14. The primary efficacy endpoint was the number of symptomatic COVID-19 cases confirmed by RT-PCR 14 days after the second dose of the vaccine. Prevention of disease severity was a major secondary efficacy endpoint, and adverse events incidence up to seven days after immunization was the primary safety outcome. The trial was registered at ClinicalTrials.gov, NCT04456595.

Findings

Between July 21 and Dec 16, 2020, 12 396 participants were enrolled and received at least one vaccine or placebo dose. There were 9,823 participants who received the two doses and were followed for at least 14 days and had, therefore, reached the final efficacy analysis. There were 253 confirmed COVID-19 cases in the cohort: 85 cases (11.0/100 person-year) among 4,953 participants in the vaccine group, and 168 cases (22·3/100 person-year) among 4,870 participants in the placebo group. The primary efficacy against symptomatic COVID-19 was 50·7% (95%CI 36·0-62·0). The

secondary efficacy against cases requiring assistance (score ≥3) and moderate and severe cases (score ≥4) were 83·7% (95%CI 58·0-93.7) and 100% (95%CI 56·4-100.0) respectively. All 6 cases of severe COVID-19 occurred in the placebo group. The incidence of adverse reactions, which was mainly pain at the administration site, was higher in the vaccine group (77·1%) than in the placebo group (66·4%). There were 67 serious adverse events reported by 64 participants and all were determined to be unrelated to vaccination, including two fatal cases. In a subset of participants, neutralizing antibody assays showed similar seroconversion and geometric mean titres against B.1.128, P.1, and P.2 variants.

Interpretation

A phase 3 clinical trial conducted in healthcare professionals in Brazil demonstrated that the inactivated CoronaVac vaccine has a good safety profile and is efficacious against any symptomatic SARS-CoV-2 infections and highly protective against moderate and severe COVID-19.

Funding: Fundação Butantan, Instituto Butantan, and São Paulo Research Foundation - FAPESP (Grants 2020/10127-1 and 2020/06409-1)

Introduction

Three coronaviruses (SARS-CoV-1, MERS, and SARS-CoV-2) have been identified as the cause of severe acute respiratory disease in humans this century. An inactivated vaccine was developed for the first of these diseases, SARS, but its development was discontinued in phase I clinical trial because the transmission receded. [1] After the emergence of COVID-19, the same group updated this development using a SARS-CoV-2 strain isolated in January 2020. The new product, later named CoronaVac (Sinovac Life Sciences, Beijing, China), had promising performance in non-clinical studies, as shown by the reduction of disease in non-human primate challenge experiments.[2] Safety and immunogenicity results in phase I/II clinical trials, in younger [3] and older adults [4], prompted the conduction of this phase III clinical trial.

Our study focused on healthcare professionals directly caring for or in close contact with COVID-19 patients. The obtention of results in a timely fashion is significant for vaccine development in a pandemic of such proportion and a a major common challenge for all COVID-19 vaccine developers. Brazil has been one of the countries most affected by the COVID-19 pandemic and overall incidence rates have reached high levels, especially in healthcare professionals caring for COVID-19 patients. Therefore, a focus on the latter group was proposed to provide a rapid means to determine the potential efficacy of a vaccine candidate.[5] This population has been shown to have higher incidence of disease in epidemiological surveys [6,7] and could, in principle, adhere better to study case surveillance. Therefore, the objective of the present phase III clinical trial was to assess the efficacy and safety of an inactivated COVID-19 vaccine in healthcare professionals. The greater number of presumed

cases and a high degree of adherence to the protocol were expected to rapidly meet the research objectives and eventual Emergency Use Authorization for CoronaVac.

Methods

Study design and participants

This is a phase III multicentre endpoint-driven, randomized, placebo-controlled clinical trial to assess the safety and efficacy of a two-dose schedule of an inactivated COVID-19 vaccine (CoronaVac, Sinovac Life Sciences, Beijing, China) containing aluminium hydroxide adjuvant in healthcare professionals ddirectly dealing with COVID-19 patients. Volunteers were recruited in sixteen clinical sites in Brazil, with 1:1 allocation ratio between vaccine and placebo. Initially, the study included only participants aged 18-59 years without previous SARS-CoV-2 infection. After phase I/II data in the elderly population became available,[4] those with 60 years of age or above were also enrolled, and a study amendment dropped any restriction of prior infection. The primary efficacy objective considered the whole study population regardless of age group and previous infection. The sample size for efficacy was calculated considering an attack rate of 2.5% and one interim analysis. The required number of cases was 61 for the interim analysis and 151 for the primary outcome analysis with estimated recruitment of 13,060 participants. The primary safety objective was incidence of adverse events by age group with up to 11800 participants in the 18-59 years group and up to 1260 in the group of 60 years or older.

Participants needed to be 18 years of age or older and work as healthcare professionals caring for COVID-19 patients and had to agree to participate by signing the informed consent form. The main exclusion criteria were pregnant or lactating

women, unstable chronic disease, previous use of any COVID-19 vaccines, and acute disease symptoms including COVID-19 in the previous 72 hours. The full protocol has been published previously.[8]

The study complied with ICH Good Clinical Practices and Brazilian ethical and regulatory guidelines, and was approved by the Brazilian National Research Ethics Council - CONEP - (CAAE 34634620.1.1001.0068) and the Brazilian National Regulatory Agency - ANVISA - (CE 47/2020) and is registered in the ClinicalTrials.gov platform (NCT0445659).

Randomization and masking

Two permuted block randomization lists were created according to age group, 18-59 years, and 60 years or older. Vaccine and placebo were randomized at a 1:1 ratio and all sites accessed the same randomization lists through an IWRS provided by Cenduit (Durham, NC, USA). Study vaccines and placebos were provided in prefilled syringes with similar characteristics. An unblinded pharmacist at each clinical site prepared the vaccine or placebo. The pharmacist only received a coded request for an experimental product and delivered the randomized product without any contact with the study participant or her/his identification information in a concealed syringe to a blind research staff. Participants and all other study staff as well as monitors, lab technicians, and data management team remained unaware of the product allocation.

Procedures

CoronaVac is an inactivated vaccine candidate against COVID-19 derived from the CN02 strain of SARS-CoV-2 grown in African green monkey kidney cells (Vero cells). At the end of the incubation period, the virus was harvested, inactivated with β-

propiolactone, concentrated, purified, and finally absorbed by aluminium hydroxide. The placebo was aluminium hydroxide diluent with no virus. Both the vaccine and placebo were prepared in a GMP-accredited facility. Vaccine (3 μg in 0.5ml) and placebo were provided in a ready-to-use syringe and administered intramuscularly following the two-dose schedule of 0,14 (+14) days. The selected vaccine doses have been proven to be sufficient for protection against SARS-CoV-2 challenge in macaques.[2]

This study was carried out in 16 clinical research centres in Brazil. All participants who provided the informed consent were enrolled after baseline assessment of inclusion and exclusion criteria, medical history, physical examination, vital signs, pregnant test, and blood tests. At screening, blood samples and a throat swab were collected for laboratory detection of SARS-CoV-2.

CoronaVac or placebo preparation was performed by the unblinded pharmacist at each site and then administered by nurses in a blinded fashion. After vaccination, safety evaluation was conducted by investigators who were unaware of treatment assignments onsite for 60 minutes. Follow-up contacts were allocated to each participant to verify the occurrence of adverse events and COVID-19 symptoms. These contacts could be made electronically, by telephone, or in-person, at the discretion of the study team and the participant informed the team about the means of contact they preferred. Contacts were made between the third and fifth day after each vaccination and thereafter every week for the first 13 weeks after vaccination and every two weeks for the remainder of the study. Once fever or other symptoms related to COVID-19 was reported, the participants were asked to seek assistance from the study team to collect a throat swab to diagnose COVID-19. All possible cases were

followed up to the resolution of all symptoms and the duration and severity of each of the signs and symptoms documented.

An independent data and safety monitoring committee was established prior to the study initiation. Safety data were assessed and reviewed by the committee to ensure safety.

Outcomes

The primary endpoint was the efficacy of CoronaVac against confirmed symptomatic COVID-19 with onset at least 14 days after the second injection in the *per protocol* population. All the cases were judged by a blind independent clinical endpoint adjudication committee. Confirmed COVID-19 cases were defined as: 1) at least two consecutive days with one or more specific symptoms (cough, newly developed taste or smell disorders, shortness of breath or dyspnea); or 2) with two or more non-specific symptoms (fever [axillary temperature ≥37·5°C], chills, sore throat, fatigue, nasal congestion or runny nose, body pain, muscle pain, headache, nausea or vomiting, diarrhoea; or 3) imaging features of COVID-19; and 4) detection of SARS-CoV-2 nucleic acid in respiratory swab by RT-PCR. A case definition based on the U.S. Food and Drug Administration (FDA) criteria was also used as a sensitivity analysis.[9]. Following the latter criteria, a positive case was considered as anyone who presented at least one of the following symptoms for two days or more, with a positive SARS-CoV-2 RT-PCR result: fever or chills, cough, shortness of breath or difficulty in breathing, fatigue, muscle or body pain, headache, sore throat, nasal congestion or runny nose, nausea or vomiting, and diarrhoea. The primary efficacy was also evaluated in distinct subgroups, including age groups, race, and ethnic group, with or without underlying medical conditions, different vaccination intervals

between two doses (<21 days or ≥21 days), and severity of COVID-19 according to WHO Clinical Progression Scale.[10] A modified intention-to treat analysis was also performed to verify the exploratory aim of evaluating the efficacy after a single dose. All the cases included for efficacy analysis had symptoms initiating up to December 16, 2020.

The primary safety endpoint was incidence of adverse reactions within 7 days after injection. The safety profile was assessed based on the safety set (SS), consisting of all the participants who received at least one dose vaccination. The events included in this analysis were those initiating up to December 16, 2020 and corresponded to a median follow-up of two months after the second dose.

Serum samples from a subset of the first participants per age group of the coordinating clinical site were analysed to determine neutralization titres by cytopathic effect-based virus neutralization test (CPE - VNT)using SARS-CoV-2 wild-type variants: B.1.128 (SARS-CoV-2 / human / BRA / SP02 / 2020 strain (MT126808.1), SARS-CoV-2-P.1 (MAN 87201 strain) and SARS-CoV-2-P.2 (LMM 38019 strain) in 96-well plates containing 5E+04 cells / mL of Vero cells (ATCC CCL-81). All procedures related to VNT were performed in a level 3 biosafety laboratory, from the Institute of Biomedical Sciences of the University of São Paulo, following WHO recommendations.

Statistical analysis

The primary efficacy analysis of was a -modified *per protocol* analysis calculated with all virologically confirmed cases of COVID-19 occurring in the period from the beginning of vaccination to two weeks after the second dose, using Cox proportional hazards regression model. This model calculates the estimated vaccine efficacy (1 -

hazard ratio), and the Wald test based on the Cox model compared to the p-values described above, and 95% confidence interval according to the appropriate alpha level was similarly transformed and presented. Cumulative incidence charts were also created with this model. The hypothesis test of the primary efficacy endpoint in the *per protocol* population was based on the on each analysis' alpha spent levels and followed up with the corresponding confidence intervals. Interim efficacy analysis was set to be triggered upon collection of at least 61 primary endpoint cases. The safety analysis included all participants who received at least one dose of CoronaVac or placebo. For neutralization assays, seroconversion was defined as a person with a post-vaccination titre ≥20 with a baseline negative result. The Geometric Mean Titres (GMT) were also calculated for those that seroconverted in each group. The Pearson Chi-square test or Fisher's exact test was adopted for the analysis of categorical outcomes. The 95% confidence intervals (95%CIs) of categorical outcomes were computed with the Clopper-Pearson method. Hypothesis testing was two-sided and P-values<0.05 was considered statistically significant.

Role of the funding sources

Employees of Fundação Butantan and Instituto Butantan participated in the study design, data collection, data analysis, data interpretation, and the report writing. Those organizations are non-profit. All the authors have full access to all the data in the study and the corresponding authors had final responsibility for the decision to submit for publication.

Results

From July 21 to December 16, 2020, 12,842 participants were screened, and 12,408 were randomized at 16 study sites in Brazil. A total of 12,396 participants received at least one dose of CoronaVac or placebo (Figure 1), 6,195 in the vaccine group and 6,201 in the placebo group.

Among those 12,396 participants, 5·1% were elderly participants aged 60 years or older, 64·2% were female, and most participants self-identified themselves as white (75·3%). More than half of the participants (55·9%) had underlying diseases, 22·5% of them were obese (BMI ≥30 kg/m²). The average age and BMI of participants were 39·5 years and 26·8 kg/m^2, respectively (Table 1).

All 12,396 participants were involved in the safety set (SS) and monitored for adverse events from the beginning of vaccination up until 12 months after the first dose vaccination. By the cut-off date, the incidence of adverse events and adverse reactions were 78·8% and 71·7%, respectively, by the cut-off date (Appendix p6). Generally, the vaccine group reported more adverse reactions than the placebo group (77·1% *vs*. 66·4%; p<0001), and most adverse reactions were solicited (73·1% *vs*. 60·0%, p<0·0001) (Figure 2A).

Among solicited adverse reactions, the incidence of local adverse reactions was 61·5% in the vaccine group, and this was higher than the 34·6% in the placebo group (p<0·0001). Local adverse reactions were mainly driven by pain at the injection site (60·3% *vs*. 32·5%, p<0·0001). All solicited local reactions were more frequently in the vaccine group, and the incidences were less than 6% in the vaccine group, except pain at the injection site (Figure 2B). Systemic adverse reactions were similar in the vaccine and placebo groups (48·4% *vs*. 47·6%, p=0·3882), including headache and

fatigue, the most common systemic symptom collected in this trial. Myalgia was more frequent in the vaccine group (11·7% *vs*. 10·5%, p=0·0257). Fever (≥37.8°C) was rare and only reported by 0·2% and 0·1% (p=0·2666) participants in the vaccine and placebo groups, respectively (Figure 2C). Unsolicited ARs were reported by 36·8% in the vaccine and 35·8% in the placebo groups (p=0·2177, Figure 2A). Only tremor, flushing and local reactions in the administration site (reported in an unsolicited period) showed higher incidence in the vaccine group. No difference was found for other unsolicited symptoms (Appendix p7-10).

In this study, 67 serious adverse events were reported by 64 participants, 33 in the vaccine group and 31 in the placebo group (Appendix p20-23). The overall incidence of SAE was 0·5%. All SAEs were determined as unrelated to the vaccine. Two deaths were reported in this trial: one case of cardiopulmonary arrest (placebo group), and one case of medication overdose (vaccine group); all of them unrelated to the vaccine. One additional death due to COVID-19 (placebo group) occurred as outcome on an ongoing case by the data cut time.

Among 9,823 participants in the *per protocol* analysis, 253 cases of symptomatic COVID-19 were reported during the primary efficacy analysis period (Table 2). There were 85 cases (11.0/100 person-year) among 4,953 participants in the vaccine group, and 168 cases (22·3/100 person-year) among 4,870 participants in the placebo group. The efficacy to prevent symptomatic COVID-19 was 50·7% (95%CI 35·9-62·0). Considering the α spending in the interim analysis, the corrected efficacy was 50.7% (95.4%CI 35·7-62·2). Sensitivity analysis of primary efficacy was conducted based on other case definitions, and the efficacy results ranged from 51·2% to 54·1% (Appendix p24).

A key secondary endpoint was to evaluate the efficacy to prevent COVID-19 disease at different clinical severities. There were 35 cases scored 3 and above, 10 cases scored 4 and above, 6 severe cases (including one fatal case) reported among the 9823 participants. For cases scored 3 and above, 5 cases were in the vaccine group, 30 were in the placebo group, resulting in a vaccine efficacy of 83·7% (95%CI 58·0-93·7). All cases scored 4 and above were in the placebo group, resulting in 100% vaccine efficacy against moderate and sever cases (95%CI 56·4-100·0).

Subgroup analyses were also conducted by the interval between two doses, the exposure status to SARS-CoV-2 pre-vaccination, age group, and underlying disease. Participants with two doses interval of fewer than 21 days showed similar efficacy (49·1%; 95%CI 33·0-61·4) as the primary efficacy analysis. For the small portion of participants who received two doses of vaccine or placebo with an interval of 21 days or more, the efficacy was calculated at 62·3% (95%CI 13·9-83·5). The efficacy was similar between different exposure status to SARS-CoV-2 pre-vaccination (Unexposed: 50·5%; Exposed: 49·5%), and between other age groups (18 to 59 years: 50·7%; ≥60 years: 51·1%). For participants with underlying diseases, a total of 130 cases were reported in this population, resulting in 48·9% efficacy (95%CI 26·6-64·5). For participants with cardiovascular disease, diabetes, and obesity, the efficacy was 39·5% (95%CI -66·4-78·0), 48·6% (95%CI -115·3-87·7) and 74·9% (95%CI 53·7-86·4), respectively. Two-hundred and fifty participants of Asian ethnicity reported 4 cases, of which 1 in the vaccine group and 3 in the placebo group, resulted in 66·0% efficacy (95%CI -226·8-96·5).

After the first dose or 14 days after the first dose, secondary efficacy endpoints were analysed using the intention-to-treat (ITT) approach. Among the 12,396 participants,

378 cases were reported after the first dose, of which 126 were in the vaccine group and 252 were in the placebo group, resulting in an efficacy of 50·8% (95%CI 39·0-60·3) after the first dose, similar to the calculated efficacy with the complete vaccination schedule. For 14 days after the first dose, 313 cases were collected among 11,431 participants, 94 were in the vaccine group and 219 were in the placebo group, resulting in an efficacy of 57·9% (95%CI 46·4-66·9) (Figure 3).

One hundred and nine participants had samples processed for neutralization assay before vaccination and two weeks after the second dose. Six of them had positive pre-vaccination samples (four for the vaccine and two for the placebo groups) and were not included in the seroconversion assessment. Two of four vaccinated participants with previous antibody titres had a 4-fold increase or higher for all tested variants. Three participants (5.2%) out of 58 in the placebo arm seroconverted for the variant B.1.1.28, but not to the other variants. Thirty-two (71.1%; GMT 64.4) of the 45 participants vaccine arm seroconverted for B.1.1.28, 31 (68.9%; GMT 46.8) for P.1, and 36 (80.0% GMT 45.8) for P.2. There were no significant differences in GMT against the B.1.128 variant as compared to P.1 GMT (p=0.34) and P.2 GMT (p=0.72).

In vaccinated individuals who seroconverted, 21 of 22 (95.5%; GMT 72.8) adults aged 18 to 59 years, 21 had seroconversion for B.1.1.28, 17 of 22 (77.3%; GMT 60.9) for P.1 and 21 of 22 (95.5%; GMT 50.4)) for P.2. Of the 23 samples analysed from participants aged 60 years or more, 11 (47.8%; GMT 58.1) evidenced seroconversion for B.1.1.28, 14 (60.9%; GMT 34.5) for P.1, and 15 (65.2%; GMT 40.0) for P.2. When the different age groups are compared, there were significant in seroconversion rates for B.1.1.28 (p<0.001) and P.2 (p=0,022) variants, but not for the P.1 variant (p=0.337). The differences in GMT between age groups were not significantly

different for the B.1.1.28 variant (p=0.086) nor the P.2 variant (p=0.174) but was different for the P.1. variant (p=0.029).

Discussion

The PROFISCOV study was designed to test CoronaVac in a group exposed to SARS-CoV-2 more often and at potentially higher infectious doses than in a community exposure. Using a smaller sample size compared to other large Phase III clinical trials with vaccine candidates, we were able to demonstrate that this vaccine was safe, well-tolerated, and efficacious. Efficacy to prevent any symptomatic COVID-19 started at 50.7% and became more extensive as disease severity increased. Of note, the case definition and professional profile of the study population allowed highly sensitive surveillance and the study was able to detect even the mildest cases of COVID-19. The conditions of this trial should be considered when the results are extrapolated to other populations or comparisons with other trials are suggested.

The vaccine performance met the requirements for Emergency Use Authorization in 32 countries and regions allowing a fast response to an ongoing public health emergency at a speed similar to other vaccine candidates receiving heavy subsidies from governments and international organizations.

One of the factors that might have affected the study's overall efficacy was the interval between two doses of 14 days. Although there were a limited number of participants in this study having doses with an interval of 21 days or higher, there was a trend to higher efficacy. Furthermore, previous neutralization data in adults were lower with a 14-days interval[3] , and, in this study, participants aged 60 years or more had a lower response than adults with the same 14-days schedule. These results contrast with previous studies where the immune responses in adults and elderly

populations with a 28-days interval schedule were comparable [3,4]. Taken together, these data suggests that it is advisable to encourage longer intervals between doses, i.e., 28 days, in the vaccine implementation. The study cannot make a clear assumption of efficacy with a single dose due to the limited number of outcomes and the odds of having more participants infected around the time of first injection in the vaccine arm (Figure 3). However, it must be noticed that the efficacy of CoronaVac was already present after the second week of the first dose.

The study was not designed to provide subgroup efficacy analysis by previous SARS-CoV-2 exposure, age group, or underlying medical conditions. Nonetheless, the efficacy found in participants with obesity is promising because this condition has been associated with lower immune response in other inactivated vaccines.[11]

There is international concern that the emergency of SARS-CoV-2 variants may alter vaccine efficacy. Two variants haveemerged in Brazil after this trial started, the so-called P.2 and P.1 Out of them, only the P.2 variant was circulating on the study centres during the period covered by this analysis. Although these variants have several mutations that are key to the function of many antibodies, there was a consistent neutralization of all these variants by serum of participants given the inactivated vaccine. This is expected as the vaccine contains the whole virus.

The observed safety and tolerability profiles were outstanding. As it was observed with other COVID-19 vaccines, no vaccine-enhanced disease effect was documented, besides post-implementation surveillance is advisable.[12] Local pain was the most frequent adverse reaction. Differences in adverse event rates between experimental and control products became an issue in several COVID-19 vaccine developments, as study blinding could be compromised leading to changes in participant behaviour.

Since CoronaVac showed similar reactogenicity to placebo, such concern was not an issue in this trial.

This pivotal trial for CoronaVac was able to demonstrate the safety and efficacy of a new COVID-19 vaccine with one of the most efficient approaches among first-wave developers maintaining the highest standards in science and ethics. After the results of this study were initially released on January 12, 2021, Butantan have delivered 38,2 million doses to the Brazilian Public Health System and Sinovac distributed additional 180 million doses in around 30 low-and-middle-income countries up to April 07, 2021. The deployment rate of this vaccine was higher and more opportune for those countries than other initiatives [13] demonstrating the success of the Sinovac-Butantan co-development and confirming that the use of traditional inactivated virus vaccine strategies cannot be ruled out as a platform of rapid public health response to epidemics or pandemics caused by emerging pathogens, such as SARS-CoV-2.

Acknowledgments

First and foremost, we would like to thank the colleagues who volunteer to participate in this trial. Not only for the scientific contribution but for also staying at the frontlines caring for COVID-19 patients and their families and friends. The technical assistance of Jorge Flores, Christopher Gast and Chistian F. Ocklenhouse from PATH was key to make this study possible. We are very grateful to the members of the Data Safety Monitoring Board and the Clinical Endpoint Adjudication Committee that generously gave their knowledge, experience, and wise advice to the study at no cost. The PROFISCOV study group is in debt for all extraordinary efforts made by our colleagues Dimas Tadeu Covas, Rui Curi, Reinaldo Noboru Sato, Raul Machado Neto, Paulo Luis Capelotto, Tiago Tadeu Rocca, Cristiano Gonçalves Pereira, Gilberto Guedes de Padua, Ricardo Haddad, Natália Lamesa Ambrósio, Luiz Rogério Perilli, and Fernanda Aparecida Orsi Castilho from Instituto Butantan and Fundação Butantan to support this study. Sandra Yi O Cho and Bruno Antônio de Oliveira, from the Regulatory Affairs Area at Butantan, deserve our special gratitude for being the most constant fellows of the clinical research team. The vendors in a clinical trial have a critical role and we were lucky to have the most dedicated staff from IQVIA (Eduardo Tedeschi, Fernanda Nobrega, Renata Toledo, Tatiana Kasteckas, Guilherme Knorst, Nicolas Rodovalho, and colleagues), Allports (José Luiz Vaz Monteiro) and WorldCourier. We were able to move ahead with the extenuating journeys required for this study just because we have the care and the patience of our loved ones.

References

1 Lin J-T, Zhang J-S, Su N, *et al.* Safety and immunogenicity from a phase I trial of inactivated severe acute respiratory syndrome coronavirus vaccine. *Antivir Ther* 2007; **12**: 1107–13.

2 Gao Q, Bao L, Mao H, *et al.* Development of an inactivated vaccine candidate for SARS-CoV-2. *Science* 2020; **369**: 77–81.

3 Zhang Y, Zeng G, Pan H, *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21**: 181–92.

4 Wu Z, Hu Y, Xu M, *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; published online Feb 3. DOI:10.1016/S1473-3099(20)30987-7.

5 Cyranoski D. China's coronavirus vaccines are leaping ahead - but face challenges as virus wanes. *Nature* 2020; **584**: 17–8.

6 Ran L, Chen X, Wang Y, Wu W, Zhang L, Tan X. Risk Factors of Healthcare Workers With Coronavirus Disease 2019: A Retrospective Cohort Study in a Designated Hospital of Wuhan in China. *Clin Infect Dis* 2020; **71**: 2218–21.

7 Pessa Valente E, Cruz Vaz da Costa Damásio L, Luz LS, da Silva Pereira MF, Lazzerini M. COVID-19 among health workers in Brazil: The silent wave. *J Glob Health* 2020; **10**: 010379.

8 Palacios R, Patiño EG, de Oliveira Piorelli R, *et al.* Double-Blind, Randomized, Placebo-Controlled Phase III Clinical Trial to Evaluate the

Efficacy and Safety of treating Healthcare Professionals with the Adsorbed COVID-19 (Inactivated) Vaccine Manufactured by Sinovac - PROFISCOV: A structured summary of a. *Trials* 2020; **21**: 853.

9 CBER/USFDA/USDHHS. Contains Nonbinding Recommendations Development and Licensure of Vaccines to Prevent COVID-19 Guidance for Industry. Silver Spring, 2020 https://www.fda.gov/regulatory-.

10 WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis* 2020; **20**: e192–7.

11 Dhakal S, Klein SL. Host Factors Impact Vaccine Efficacy: Implications for Seasonal and Universal Influenza Vaccine Programs. *J Virol* 2019; **93**. DOI:10.1128/JVI.00797-19.

12 Munoz FM, Cramer JP, Dekker CL, *et al.* Vaccine-associated enhanced disease: Case definition and guidelines for data collection, analysis, and presentation of immunization safety data. *Vaccine* 2021; published online Feb 23. DOI:10.1016/j.vaccine.2021.01.055.

13 COVAX. COVAX reaches over 100 economies, 42 days after first international delivery. 2021. https://www.gavi.org/news/media-room/covax-reaches-over-100-economies-42-days-after-first-international-delivery (accessed April 8, 2021).

Figure legends

Figure 1: Study Profile.

All participants enrolled from Jul. 21 to Dec. 16, 2020, were shown in the diagram.

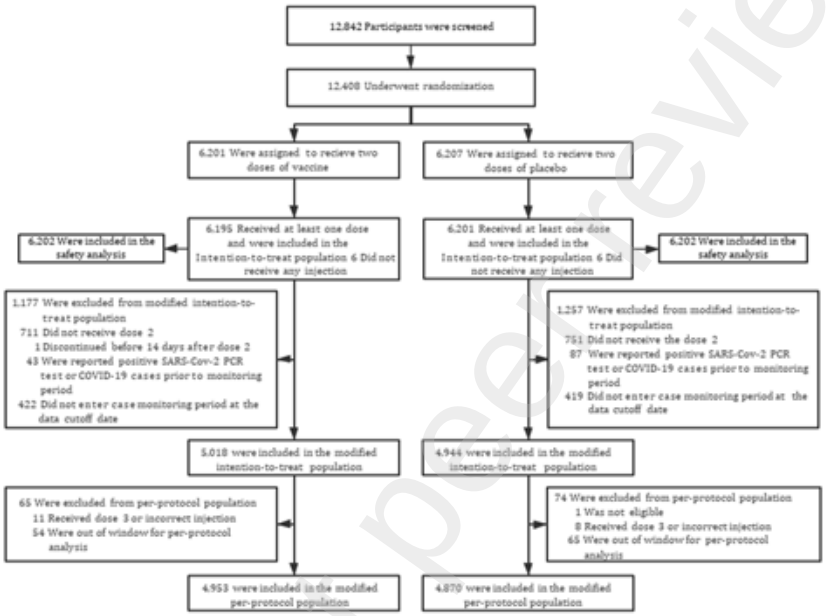


Figure 2: Overview of Adverse Reactions and Solicited Local/Systemic Adverse Reactions.

The percentage of participants who had adverse reactions after any administration of vaccine or placebo was shown. (A) The overview of the percentage of participants who had any adverse reactions; (B) The percentage of participants who had local solicited adverse reactions by different symptoms; (C) The percentage of participants who had systematic solicited adverse reactions by different symptoms.

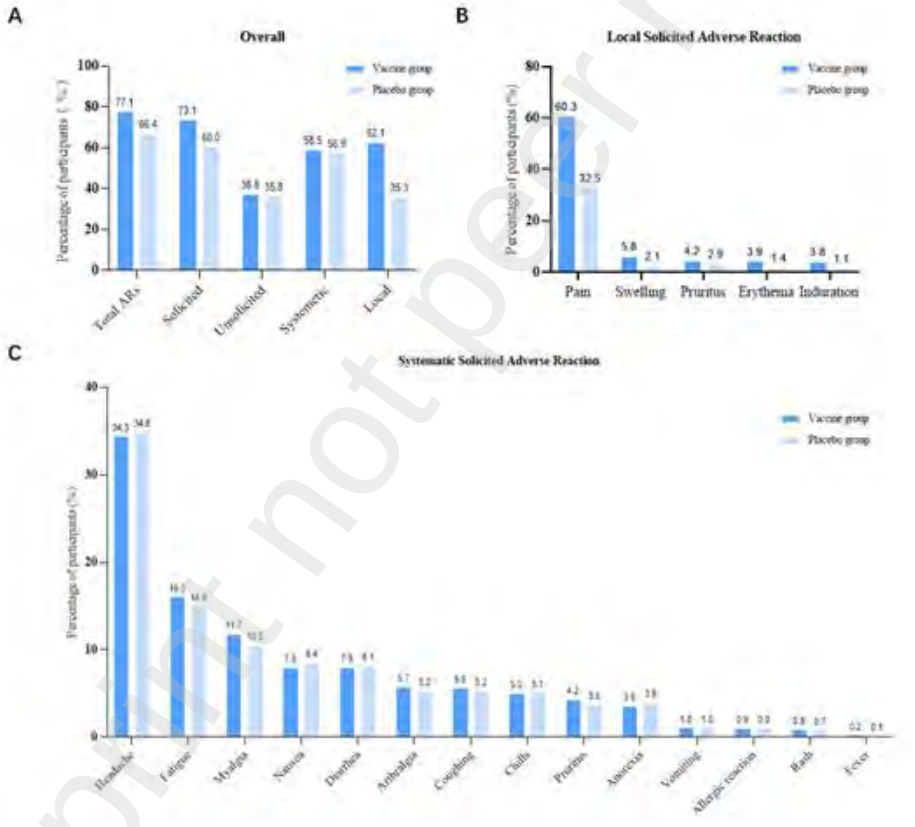
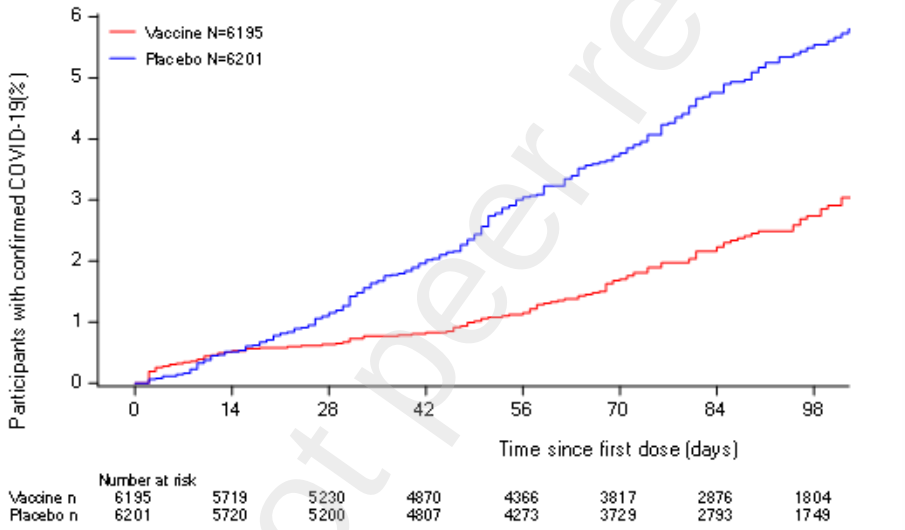


Figure 3. Efficacy of vaccine against COVID-19 cases after the 1st dose and the Kaplan-Meier cumulative incidence curves

(A) The Kaplan-Meier cumulative incidence curves of symptomatic Covid-19 cases after the 1st dose of vaccination. (B) The number of cases collected, incidence density, and efficacy of 14 days after the 1st dose and 2nd dose. Analysis was based on the intention-to-treat population; Incidence density: per 100 person-years.

A



B

Time	No. of cases	Vaccine n/N(incidence density)	Placebo n/N(incidence density per 100 person-year)	Efficacy (95%CI)
14 days after 1st dose	313	94/5717(8·0)	219/5714(19·0)	57·9 (46·4, 66·9)
14 days after 2nd dose	253	85/4953(11·0)	168/4870(22·3)	50·7 (35·9, 62·0)

Tables

Table 1: Baseline characteristics of participants who received at least one dose of vaccine or placebo

	Vaccine (N=6195)	Placebo (N=6201)	Total (N=12396)
Age Group			
18~59 years	5879 (94·9%)	5885 (94·9%)	11764 (94·9%)
≥60 years	316 (5·1%)	316 (5·1%)	632 (5·1%)
Gender			
Male	2270 (36·6%)	2171 (35·0%)	4441 (35·8%)
Female	3925 (63·4%)	4030 (65·0%)	7955 (64·2%)
Ethnic			
White	4685 (75·8%)	4633 (74·8%)	9318 (75·3%)
Multiracial	1012 (16·4%)	1065 (17·2%)	2077 (16·8%)
Black or African American	329 (5·3%)	319 (5·2%)	648 (5·2%)
Asian	148 (2·4%)	163 (2·6%)	311 (2·5%)
American Indian or Alaska Native	11 (0·2%)	13 (0·2%)	24 (0·2%)

	Vaccine (N=6195)	Placebo (N=6201)	Total (N=12396)
Underlying Disease	3441 (55·5%)	3484 (56·2%)	6925 (55·9%)
Cardiovascular disease	792 (12·8%)	773 (12·5%)	1565 (12·6%)
Diabetes	218 (3·5%)	197 (3·2%)	415 (3·4%)
Obesity	1386 (22·4%)	1403 (22·6%)	2789 (22·5%)
Age, years	39·42 (10·7)	39·59 (10·8)	39·50 (10·8)
BMI, kg/m^2	26·841 (5·1)	26·792 (5·3)	26·817 (5·2)

Data are n (%) and mean (SD).

Table 2. Efficacy against COVID-19 cases 14 days after the 2nd dose

	Total No. of cases	Vaccine n/N(incidence density)	Placebo n/N(incidence density per 100 person-year)	Vaccine Efficacy (95%CI)
Overall	253	85/4953(11·0)	168/4870(22·3)	50·7 (35·9, 62·0) [1]
Severity				
Score 3 and above	35	5/4953(0·7)	30/4870 (4·1)	83·7(58·0, 93·7)
Score 4 and above	10	0/4953 (0·0)	10/4870 (1·4)	100·0(56·4, 100·0) [2]
Severe	6	0/4953 (0·0)	6/4870 (0·8)	100·0(16·9, 100·0) [2]
Interval between two doses				
<21 days	226	77/4184(11·6)	149/4148(22·7)	49·1(33·0, 61·4)
≥21 days	27	8/769(8·6)	19/722(23·1)	62·3(13·9, 83·5)

	Total No. of cases	Vaccine	Placebo	Vaccine Efficacy (95%CI)
		n/N(incidence density)	n/N(incidence density per 100 person-year)	
Exposure to SARS-Cov-2 pre-vaccination				
Unexposed	200	67/3637(13·3)	133/3587(26·8)	50·5(33·6, 63·1)
Exposed	9	3/401(5·9)	6/408(11·7)	49·5(-101·8, 87·4)
Age group				
18~59 years	247	83/4741 (11·3)	164/4663 (22·8)	50·7(35·8, 62·1)
≥60 years	6	2/212 (10·8)	4/207 (21·9)	51·1(-166·9, 91·0)
Underlying Disease				
No	123	41/2222(13·2)	82/2140(27·8)	52·4(30·8, 67·3)
Yes	130	44/2731(10·6)	86/2730(20·8)	48·9(26·6, 64·5)

	Total No. of cases	Vaccine n/N(incidence density)	Placebo n/N(incidence density per 100 person-year)	Vaccine Efficacy (95%CI)
Cardiovascular disease	16	6/621(7·1)	10/608(11·6)	39·5(-66·4, 78·0)
Diabetes	8	3/175(11·2)	5/159(21·1)	48·6(-115·3, 87·7)
Obesity	63	13/1099(5·8)	50/1112(23·0)	74·9(53·7, 86·4)
Asian	4	1/125(5.38)	3/125(15.54)	66.02(-226.82, 96.47)

[1] The efficacy corrected based on the α spending in the interim analysis was 50.7% (95.4%CI: 35.7, 62.2).

[2] Calculated based on Poisson regression model

Appendix 1 Protocol violation

Table 1-1. Data set division of each protocol violation

No.	Protocol Violations	Efficacy Evaluation			Safety Evaluation		
		PPS	ITT	mITT	SS	SS1	SS2
1	Not vaccinated after randomisation	N	N	N	N	N	N
2	Received 1 dose vaccination	N	Y	N	Y	Y	N
3	Withdraw before 14 days after the second dose vaccination	N	Y	N	NA	NA	NA
4	Received 3 doses vaccination	N	Y	Y	Y	Y	Y
5	Participated in any COVID-19 vaccine clinical trial or vaccinated COVID-19 vaccine in the past	N	Y	Y	NA	NA	NA
6	Received the second dose vaccination beyond the window period	N	Y	Y	Y	Y	Y
7	Received wrong vaccine*	N	Y	Y	NA	NA	NA
8	The time of data analysis was before 14 days after the second dose vaccination	N	Y	N	NA	NA	NA
9	PCR positive between the first dose vaccination to the 14 days after the second dose vaccination	N	Y	Y	NA	NA	NA
10	Diagnosed COVID-19 between the first dose vaccination to the 14 days after the second dose vaccination	N	Y	Y	NA	NA	NA

*Details see Table 1-2.

Table 1-2. List of wrong vaccinations*

No. of subject	Wrong dose vaccination	No. of vaccine	Date of wrong dose vaccination	Describe of protocol violation
111451	1	111454	2020/8/6	
111577	2	111571	2020/8/25	
112384	1	112386	2020/8/20	
112538	2	114579	2020/9/4	
112828	2	111828	2020/9/8	
113046	2	113007	2020/9/9	
115170	2	115191	2020/9/23	
115191	2	115170	2020/9/23	
116623	1	116593	2020/9/17	
116737	2	wrong arm**	2020/10/1	Due to the error of the unblinded pharmacist, subject 116737 was assigned the wrong vaccine in V2.
116811	1	wrong arm**	2020/9/18	Due to the error of the unblinded pharmacist, subject 116811 was assigned the wrong vaccine in V1.
116881	1	wrong arm**	2020/9/18	Due to lack of supervision, the unblinded pharmacist assigned the wrong vaccine to subject 116881 in V1.
117927	2	118063	2020/10/9	
118339	1	wrong arm**	2020/9/26	Due to the error of the unblinded staff, an error occurred in the allocation of vaccine to subject 118339. Date of occurrence of PD: 2020-09-26
119167	2	119538	2020/10/20	
119278	1	wrong arm**	2020/10/3	Due to the absence of double review, subject 119278 was assigned the wrong vaccine in V1.

120446	1	120426	2020/11/6	
120579	1	Unknown**	2020/10/19	The unblinded monitor confirmed that subject 120579 was vaccinated on October 19, 2020, but the IWRS indicated that this assignment did not occur on that day. Therefore, it is unknown which vaccine the subject has been assigned.

*From the protocol deviation list provided by the monitor

**In the overall and corresponding dose safety analysis, from a conservative perspective, subjects with “wrong arm” and “unknown” are analyzed by vaccine group.

Appendix 2 Study sites

Table 2. Information of study sites

Code.	Study Site	Address	Principal Investigator
SAO06	Instituto de Infectologia Emílio Ribas	Sao Paulo, SP, Brazil, 01246-900	Luiz Carlos Pereira Júnior, MD, PhD
CWB01	Hospital das Clínicas da Universidade Federal do Paraná	Curitiba, PR, Brazil, 80060-900	Sonia Mara Raboni, MD, PhD
POA01	Hospital São Lucas da Pontificia Universidade Catolica do Rio Grande do Sul	Porto Alegre, RS, Brazil, 90619-900	Fabiano Ramos, MD, PhD
BHZ01	Universidade Federal de Minas Gerais	Belo Horizonte, MG, Brazil, 30750-140	Mauro Martins Teixeira, MD, PhD
BSB01	Universidade de Brasília	Brasilia, DF, Brazil, 71691-082	Gustavo Adolfo Sierra Romero, MD, PhD
SCS01	Universidade Municipal de São Caetano do Sul	São Caetano do Sul, SP, Brazil, 09521-160	Fábio Eudes Leal, MD, PhD
SAO06	Instituto Israelita de Ensino e Pesquisa Albert Einstein	Sao Paulo, SP, Brazil, 05652-900	Luis Fernando Aranha Camargo, MD, PhD
VCP01	Hospital das Clínicas da UNICAMP	Campinas, SP, Brazil, 13083-888	Francisco Hideo Aoki, MD, PhD
RAO01	Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo	Ribeirao Preto, SP, Brazil, 14015-069	Eduardo Barbosa Coelho, MD, PhD
SAO01	Centro de Pesquisas Clínicas do Instituto Central do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo	Sao Paulo, SP, Brazil, 05403-000	Esper Georges Kallás, MD,PhD
PET01	Universidade Federal de Pelotas, Faculdade de Medicina. Departamento de Clínica Médica	Pelotas, RS, Brasil, 96030-002	Danise Senna Oliveira, MD, PhD
SJP01	Faculdade de Medicina de São José do Rio Preto - FAMERP	São José Do Rio Preto, SP, Brazil, 15090-000	Maurício Lacerda Nogueira, MD, PhD
CWB01	Universidade Federal de Mato Grosso, Faculdade de Ciências Médicas, Hospital Univeristário Júlio Müller.	Cuiabá, MT – Brasil, 78048-902	Cor Jesus Fernandes Fontes, MD, PhD
BAT01	Hospital de Amor	Barretos, SP, Brazil 14780-000	Gecilmara Cristina Salviato Pileggi, MD, PhD
CGR01	Hospital Universitário Maria Aparecida Pedrossian, Universidade Federal de Mato Grosso do Sul	Campo Grande, MS, Brazil, 79080-190	Ana Lúcia Lyrio de Oliveira, MD, PhD

RIO01	Instituto de Infectologia Evandro Chagas - Fiocruz	Rio De Janeiro, Brazil, 21710-232	André Machado de Siqueira, MD, PhD

Appendix 3 Adverse Events

Table 3-1. Overview of adverse events in subjects after vaccination

Category	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Total AEs	29041	5096(82·2%)	25619	4670(75·4%)	54660	9766(78·8%)	<0·0001
AEs related to vaccine	21162	4782(77·1%)	17270	4111(66·4%)	38432	8893(71·7%)	<0·0001
Solicited AEs	14949	4536(73·1%)	11119	3714(60·0%)	26068	8250(66·6%)	<0·0001
Unsolicited AEs	6213	2284(36·8%)	6151	2215(35·8%)	12364	4499(36·3%)	0·2177
Systemic AEs	14164	3625(58·5%)	14056	3525(56·9%)	28220	7150(57·7%)	0·0842
Local AEs	6998	3854(62·1%)	3213	2188(35·3%)	10211	6042(48·7%)	<0·0001
AEs within 60 min	611	460(7·4%)	525	413(6·7%)	1136	873(7·0%)	0·1064
AEs within 0-7 days	16583	4613(74·4%)	12625	3823(61·7%)	29208	8436(68·1%)	<0·0001
AEs in 8-28 days	4046	1619(26·1%)	4132	1615(26·1%)	8178	3234(26·1%)	0·9837
Grade 1 Adverse Event	17693	4652(75·0%)	13889	3901(63·0%)	31582	8553(69·0%)	<0·0001
Grade 2 Adverse Event	3306	1648(26·6%)	3158	1546(25·0%)	6464	3194(25·8%)	0·042
Grade 3 Adverse Event	144	98(1·6%)	205	128(2·1%)	349	226(1·8%)	0·0441
AEs unrelated to vaccine	7813	2398(38·7%)	8295	2442(39·4%)	16108	4840(39·0%)	0·3869

Table 3-2. Adverse reactions reported within 28 days after whole-schedule vaccination

Category	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Total adverse reactions	21162	4782(77·1%)	17270	4111(66·4%)	38432	8893(71·7%)	<0·0001
Solicited adverse reactions	14949	4536(73·1%)	11119	3714(60·0%)	26068	8250(66·6%)	<0·0001
Local adverse reactions	6767	3815(61·5%)	3074	2143(34·6%)	9841	5958(48·1%)	<0·0001
Vaccination site pain	5508	3742(60·3%)	2555	2014(32·5%)	8063	5756(46·4%)	<0·0001
Swelling	434	359(5·8%)	147	130(2·1%)	581	489(3·9%)	<0·0001
Pruritus	306	263(4·2%)	207	181(2·9%)	513	444(3·6%)	<0·0001
Redness	264	241(3·9%)	93	89(1·4%)	357	330(2·7%)	<0·0001
Induration	255	235(3·8%)	72	67(1·1%)	327	302(2·4%)	<0·0001
Systemic adverse reactions	8182	2999(48·4%)	8045	2947(47·6%)	16227	5946(48·0%)	0·3882
Headache	3034	2128(34·3%)	3098	2157(34·8%)	6132	4285(34·6%)	0·5583
Fatigue	1209	989(16·0%)	1164	922(14·9%)	2373	1911(15·4%)	0·1059
Myalgia	879	727(11·7%)	771	648(10·5%)	1650	1375(11·1%)	0·0257
Nausea	573	490(7·9%)	629	522(8·4%)	1202	1012(8·2%)	0·2939
Diarrhea	576	492(7·9%)	576	501(8·1%)	1152	993(8·0%)	0·7659
Arthralgia	411	353(5·7%)	369	321(5·2%)	780	674(5·4%)	0·2195
Cough	392	343(5·5%)	369	322(5·2%)	761	665(5·4%)	0·4254

Category	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Chills	359	309(5·0%)	350	313(5·1%)	709	622(5·0%)	0·8693
Pruritus	315	263(4·2%)	266	225(3·6%)	581	488(3·9%)	0·0874
Appetite impaired	241	217(3·5%)	268	243(3·9%)	509	460(3·7%)	0·2169
Vomiting	64	61(1·0%)	66	61(1·0%)	130	122(1·0%)	1·0000
Hypersensitivity	66	58(0·9%)	68	58(0·9%)	134	116(0·9%)	1·0000
Rash	53	49(0·8%)	47	42(0·7%)	100	91(0·7%)	0·5281
Fever	10	9(0·2%)	4	4(0·1%)	14	13(0·1%)	0·2666
Unsolicited adverse reactions	6213	2284(36·8%)	6151	2215(35·8%)	12364	4499(36·3%)	0·2177
Tremor	10	10(0·2%)	1	1(0·0%)	11	11(0·1%)	0·0117
Complex local pain syndrome	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Wheezing	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Vaccination site pain	133	124(2·0%)	70	65(1·1%)	203	189(1·5%)	<0·0001
Vaccination site redness	19	17(0·3%)	10	10(0·2%)	29	27(0·2%)	0·2473
Vaccination site swelling	16	15(0·2%)	6	6(0·1%)	22	21(0·2%)	0·0781
Oedema	14	14(0·2%)	6	6(0·1%)	20	20(0·2%)	0·1150
Vaccination site induration	18	17(0·3%)	3	3(0·1%)	21	20(0·2%)	0·0026
Vaccination site warmth	10	10(0·2%)	5	5(0·1%)	15	15(0·1%)	0·3015

Category	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Oedema peripheral	4	4(0·1%)	1	1(0·0%)	5	5(0·0%)	0·3749
Intestinal angina	5	5(0·1%)	3	3(0·1%)	8	8(0·1%)	0·7265
Paraesthesia oral	6	6(0·1%)	1	1(0·0%)	7	7(0·1%)	0·1249
Gastritis	4	4(0·1%)	2	2(0·0%)	6	6(0·1%)	0·6874
Abdominal pain lower	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Gastroesophageal reflux disease	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Muscular weakness	5	5(0·1%)	3	3(0·1%)	8	8(0·1%)	0·7265
Joint swelling	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Ecchymosis	5	5(0·1%)	2	2(0·0%)	7	7(0·1%)	0·4530
Petechiae	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Alopecia	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Sinusitis	7	7(0·1%)	4	4(0·1%)	11	11(0·1%)	0·5486
Flushing	39	37(0·6%)	20	18(0·3%)	59	55(0·4%)	0·0142
Hyperaemia	13	13(0·2%)	10	8(0·1%)	23	21(0·2%)	0·3829
Hypoacusis	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Photophobia	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Anxiety disorder	5	4(0·1%)	2	2(0·0%)	7	6(0·1%)	0·6874

Category	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Tachycardia	7	7(0·1%)	4	4(0·1%)	11	11(0·1%)	0·5486
Palpitations	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000

Table 3-3. Adverse reactions reported within 14 days after first dose vaccination

Category	Vaccine group (N=6196)		Placebo group (N=6200)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Total adverse reactions	11658	4058(65·5%)	9964	3438(55·5%)	21622	7496(60·5%)	<0·0001
Local adverse reactions							
Vaccination site pain	2890	2750(44·4%)	1442	1387(22·4%)	4332	4137(33·4%)	<0·0001
Induration	90	88(1·4%)	35	34(0·6%)	125	122(1·0%)	<0·0001
Swelling	185	162(2·6%)	77	72(1·2%)	262	234(2·0%)	<0·0001
Redness	97	95(1·5%)	52	48(0·8%)	149	143(1·2%)	<0·0001
Pruritus	154	147(2·4%)	133	126(2·0%)	287	273(2·2%)	0·1993
Warmth	6	6(0·1%)	2	2(0·0%)	8	8(0·1%)	0·1794
Rash	5	4(0·1%)	2	2(0·0%)	7	6(0·1%)	0·4529
Systemic adverse reactions							
Fever	8	7(0·1%)	8	8(0·1%)	16	15(0·1%)	1·0000
Hypersensitivity	53	47(0·8%)	50	44(0·7%)	103	91(0·7%)	0·7537
Rash	42	36(0·6%)	32	30(0·5%)	74	66(0·5%)	0·4625
Diarrhea	502	451(7·3%)	512	454(7·3%)	1014	905(7·3%)	0·9450
Appetite impaired	208	188(3·0%)	231	213(3·4%)	439	401(3·2%)	0·2230

Category	Vaccine group (N=6196)		Placebo group (N=6200)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Vomiting	48	47(0·8%)	51	49(0·8%)	99	96(0·8%)	0·9185
Nausea	464	423(6·8%)	521	445(7·2%)	985	868(7·0%)	0·4599
Myalgia	686	604(9·8%)	631	545(8·8%)	1317	1149(9·3%)	0·0677
Headache	2615	1944(31·4%)	2726	1996(32·2%)	5341	3940(31·8%)	0·3348
Cough	380	337(5·4%)	364	318(5·1%)	744	655(5·3%)	0·4458
Fatigue	1016	860(13·9%)	943	798(12·9%)	1959	1658(13·4%)	0·1018
Arthralgia	331	293(4·7%)	308	276(4·5%)	639	569(4·6%)	0·4659
Chills	274	252(4·1%)	285	266(4·3%)	559	518(4·2%)	0·5596
Pruritus	243	213(3·4%)	226	194(3·1%)	469	407(3·3%)	0·3387
Oedema	8	8(0·1%)	3	3(0·1%)	11	11(0·1%)	0·1457
Chest pain	7	7(0·1%)	4	4(0·1%)	11	11(0·1%)	0·3873
Warm at the vaccination site	6	6(0·1%)	2	2(0·0%)	8	8(0·1%)	0·1794
Rash at the vaccination site	5	4(0·1%)	2	2(0·0%)	7	6(0·1%)	0·4529
Tremor	8	8(0·1%)	1	1(0·0%)	9	9(0·1%)	0·0214
Paraesthesia oral	5	5(0·1%)	1	1(0·0%)	6	6(0·1%)	0·1248
Lower abdominal pain	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	0·6248

Category	Vaccine group (N=6196)		Placebo group (N=6200)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Gastritis	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	0·6248
Back pain	26	26(0·4%)	19	17(0·3%)	45	43(0·4%)	0·1733
Muscle spasms	4	4(0·1%)	2	2(0·0%)	6	6(0·1%)	0·4529
Muscular weakness	3	3(0·1%)	1	1(0·0%)	4	4(0·0%)	0·3748
Hyperhidrosis	12	12(0·2%)	7	7(0·1%)	19	19(0·2%)	0·2627
Ecchymosis	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	0·6248
Alopecia	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	0·6248
Oral herpes	16	16(0·3%)	10	9(0·2%)	26	25(0·2%)	0·1681
Rhinitis	5	5(0·1%)	3	3(0·1%)	8	8(0·1%)	0·5075
Conjunctivitis	4	4(0·1%)	2	2(0·0%)	6	6(0·1%)	0·4529
Sinusitis	4	4(0·1%)	1	1(0·0%)	5	5(0·0%)	0·2185
Amygdalitis	2	2(0·0%)	2	1(0·0%)	4	3(0·0%)	0·6248
Flushing	18	18(0·3%)	13	12(0·2%)	31	30(0·2%)	0·2803
Palpitation	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	0·6248

Table 3-4. Adverse reactions reported within 28 days after second-dose vaccination

Category	Vaccine group (N=5453)		Placebo group (N=5481)		Total (N=10934)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Total adverse reactions	9481	3294(60·1%)	7329	2418(44·3%)	16810	5712(52·2%)	<0·0001
Local adverse reactions							
Vaccination site pain	2746	2520(46·0%)	1188	1079(19·8%)	3934	3599(32·9%)	**<0·0001**
Induration	180	174(3·2%)	40	39(0·7%)	220	213(2·0%)	**<0·0001**
Swelling	265	235(4·3%)	76	70(1·3%)	341	305(2·8%)	**<0·0001**
Redness	186	174(3·2%)	51	51(0·9%)	237	225(2·1%)	**<0·0001**
Pruritus	174	154(2·9%)	109	89(1·6%)	283	243(2·2%)	**<0·0001**
Sclerosis at the vaccination site	2	2(0·0%)	0	0(0·0%)	2	2(0·0%)	0·5000
Epidermis exfoliation at the vaccination site	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Pustules at the vaccination site	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Systemic adverse reactions							
Fever	3	3(0·1%)	4	4(0·1%)	7	7(0·1%)	0·7258
Hypersensitivity	37	32(0·6%)	43	37(0·7%)	80	69(0·6%)	0·5482
Rash	25	25(0·5%)	25	23(0·4%)	50	48(0·4%)	0·8852
Diarrhea	335	300(5·5%)	340	296(5·4%)	675	596(5·5%)	0·9329

Category	Vaccine group (N=5453)		Placebo group (N=5481)		Total (N=10934)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Appetite impaired	126	110(2·0%)	143	131(2·4%)	269	241(2·2%)	0·1714
Vomiting	50	50(0·9%)	48	45(0·8%)	98	95(0·9%)	0·6805
Nausea	304	263(4·8%)	311	266(4·9%)	615	529(4·8%)	0·8586
Myalgia	526	439(8·0%)	478	403(7·4%)	1004	842(7·7%)	0·2365
Headache	1957	1354(24·7%)	1922	1317(24·2%)	3879	2671(24·4%)	0·5044
Cough	283	247(4·5%)	282	245(4·5%)	565	492(4·5%)	1·0000
Fatigue	593	496(9·1%)	636	538(9·9%)	1229	1034(9·5%)	0·1504
Arthralgia	229	187(3·4%)	202	178(3·3%)	431	365(3·3%)	0·6706
Chills	185	164(3·0%)	200	186(3·4%)	385	350(3·2%)	0·232
Pruritus	155	129(2·4%)	117	100(1·8%)	272	229(2·1%)	0·0615
Oedema	6	6(0·1%)	3	3(0·1%)	9	9(0·1%)	0·5076
Complex local pain syndrome	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Intestinal angina	3	3(0·1%)	1	1(0·0%)	4	4(0·0%)	0·6249
Gastritis	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Pain in limb	29	25(0·5%)	18	15(0·3%)	47	40(0·4%)	0·1532
Neck pain	11	11(0·2%)	5	5(0·1%)	16	16(0·2%)	0·2098

Category	Vaccine group (N=5453)		Placebo group (N=5481)		Total (N=10934)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Dyspnea	19	18(0·3%)	10	10(0·2%)	29	28(0·3%)	0·1844
Rhinallergosis	8	8(0·2%)	5	5(0·1%)	13	13(0·1%)	0·5808
Erythema	36	35(0·6%)	25	23(0·4%)	61	58(0·5%)	0·1470
Ecchymosis	3	3(0·1%)	1	1(0·0%)	4	4(0·0%)	0·6249
Skin warm	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Pharyngitis	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Flushing	21	20(0·4%)	7	7(0·1%)	28	27(0·3%)	0·0190
Hyperaemia	6	6(0·1%)	5	4(0·1%)	11	10(0·1%)	0·7538
Eye irritation	4	4(0·1%)	3	2(0·0%)	7	6(0·1%)	0·6874
Anxiety disorder	5	4(0·1%)	1	1(0·0%)	6	5(0·1%)	0·3749
Tachycardia	5	5(0·1%)	2	2(0·0%)	7	7(0·1%)	0·4530

Table 3-5. Adverse events in subjects with concomitant diseases

Concomitant disease	Vaccine group (N=3447)		Placebo group (N=3478)		Total (N=6925)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Cardiovascular disease	2553	560/794(70·5%)	2083	480/771(62·3%)	4636	1040/1565(66·5%)	0·0006
Diabetes	802	150/219(68·5%)	554	123/196(62·8%)	1356	273/415(65·8%)	0·2543
Obesity	5147	1058/1388(76·2%)	4171	933/1401(66·6%)	9318	1991/2789(71·4%)	<0·0001
Chronic lung disease	7	4/5(80·0%)	2	1/4(25·0%)	9	5/9(55·6%)	0·2063
Malignant disease	85	19/27(70·4%)	87	18/25(72·0%)	172	37/52(71·2%)	1·0000

Table 3-6. Adverse reactions in subjects with concomitant diseases

Category	Vaccine group (N=3447)		Placebo group (N=3478)		Total (N=6925)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Total adverse reactions	12974	2701(78·4%)	10961	2413(69·4%)	23935	5114(73·9%)	<0·0001
Solicited adverse reactions	9046	2562(74·3%)	6962	2176(62·6%)	16008	4738(68·4%)	<0·0001
Local adverse reactions	3935	2134(61·9%)	1836	1235(35·5%)	5771	3369(48·7%)	<0·0001
Vaccination site pain	3143	2096(60·8%)	1512	1156(33·2%)	4655	3252(47·0%)	<0·0001
Swelling	277	225(6·5%)	96	84(2·4%)	373	309(4·5%)	<0·0001
Redness	156	141(4·1%)	55	52(1·5%)	211	193(2·8%)	<0·0001
Induration	162	147(4·3%)	43	38(1·1%)	205	185(2·7%)	<0·0001
Vaccination site pruritus	197	163(4·7%)	130	113(3·3%)	327	276(4·0%)	0·0017
Systemic adverse reactions	5111	1764(51·2%)	5126	1761(50·6%)	10237	3525(50·9%)	0·6653
Headache	1813	1241(36·0%)	1927	1297(37·3%)	3740	2538(36·7%)	0·2725
Fatigue	784	620(18·0%)	752	588(16·9%)	1536	1208(17·5%)	0·2414
Myalgia	552	448(13·0%)	502	417(12·0%)	1054	865(12·5%)	0·2165
Nausea	343	294(8·5%)	410	337(9·7%)	753	631(9·1%)	0·0950
Diarrhea	370	312(9·1%)	352	306(8·8%)	722	618(8·9%)	0·7360
Arthralgia	270	225(6·5%)	255	221(6·4%)	525	446(6·4%)	0·7693
Pruritus	210	167(4·8%)	174	145(4·2%)	384	312(4·5%)	0·1829

Category	Vaccine group (N=3447)		Placebo group (N=3478)		Total (N=6925)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Cough	263	226(6·6%)	236	202(5·8%)	499	428(6·2%)	0·2121
Chills	233	197(5·7%)	216	189(5·4%)	449	386(5·6%)	0·6374
Appetite impaired	150	132(3·8%)	171	154(4·4%)	321	286(4·1%)	0·2271
Rash	31	28(0·8%)	36	32(0·9%)	67	60(0·9%)	0·6978
Hypersensitivity	47	41(1·2%)	50	40(1·2%)	97	81(1·2%)	0·9113
Vomiting	40	38(1·1%)	44	39(1·1%)	84	77(1·1%)	1·0000
Fever	5	5(0·2%)	1	1(0·0%)	6	6(0·1%)	0·1232
Unsolicited adverse reactions	3928	1396(40·5%)	3999	1364(39·2%)	7927	2760(39·9%)	0·2802

Appendix 4 Serious Adverse Events

Table 4. Serious Adverse Events by System Organ Class/Preferred Term

SAE	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Overall SAE	34	33(0·5%)	33	31(0·5%)	67	64(0·5%)	0·9004
Infection and infestations	13	13(0·2%)	14	13(0·2%)	27	26(0·2%)	1·0000
COVID-19	2	2(0·0%)	9	9(0·2%)	11	11(0·1%)	0·0384
Appendicitis	5	5(0·1%)	1	1(0·0%)	6	6(0·1%)	0·2186
Pyelonephritis	2	2(0·0%)	2	2(0·0%)	4	4(0·0%)	1·0000
Severe acute respiratory syndrome (SARS)	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Vestibular neuronitis	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Urinary tract infection	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Diverticulitis	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Pelvic inflammatory disease	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Nasal abscess	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Injury, poisoning and procedural complications	4	4(0·1%)	5	5(0·1%)	9	9(0·1%)	0·7537
Road traffic accident	1	1(0·0%)	2	2(0·0%)	3	3(0·0%)	0·6247
Limb injury	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000

SAE	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Foot fracture	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Fall	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Ankle fracture	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Fracture	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Sacroiliac fracture	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Psychiatric disorders	3	3(0·1%)	2	2(0·0%)	5	5(0·0%)	1·0000
Suicidal ideation	2	2(0·0%)	0	0(0·0%)	2	2(0·0%)	0·5000
Bipolar disorder	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Suicide attempt	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Alcohol abuse	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Pregnancy, puerperium and perinatal conditions	1	1(0·0%)	3	3(0·1%)	4	4(0·0%)	0·3746
Abortion	1	1(0·0%)	2	2(0·0%)	3	3(0·0%)	0·6247
Foetal death	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
General disorders and administration site conditions	3	3(0·1%)	0	0(0·0%)	3	3(0·0%)	0·2499
Systemic inflammatory response syndrome	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Death	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000

SAE	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Chest pain	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Musculoskeletal and connective tissue disorders	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Arthralgia	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Intervertebral disc disorder	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Intervertebral disc protrusion	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Respiratory, thoracic and mediastinal disorders	3	3(0·1%)	0	0(0·0%)	3	3(0·0%)	0·2499
Dyspnea	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Asthma	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Acute pulmonary oedema	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Nervous system disorders	1	1(0·0%)	1	1(0·0%)	2	2(0·0%)	1·0000
Syncope	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Transient ischaemic attack	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Renal and urinary disorders	0	0(0·0%)	2	2(0·0%)	2	2(0·0%)	0·2497
Nephrolithiasis	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Obstructive nephropathy	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Gastrointestinal disorders	1	1(0·0%)	1	1(0·0%)	2	2(0·0%)	1·0000

SAE	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Abdominal pain	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Haemorrhoids thrombosed	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Vascular disorders	2	2(0·0%)	0	0(0·0%)	2	2(0·0%)	0·5000
Deep vein thrombosis	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Hypertension	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Metabolism and nutrition disorders	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Hypokalaemia	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Cardiac disorders	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Cardio-respiratory arrest	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Reproductive system and breast disorders	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Endometriosis	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Skin and subcutaneous tissue disorders	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Rash	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Hepatobiliary disorders	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Cholelithiasis	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997

Appendix 5 Efficacy Analysis

Table 5-1. Efficacy analysis by case definitions

Case definition	Total No. of cases	Vaccine	Placebo	Vaccine Efficacy (95%CI)
		n/N(incidence density)	n/N(incidence density per 100 person-year)	
Case definition 1	253	85/4953(11·0)	168/4870(22·3)	50·7 (35·9, 62·0)
Case definition 2	261	87/4953(11·1)	174/4870(22·8)	51·2(36·9, 62·3)
Case definition 3	250	80/4953(10·4)	170/4870(22·7)	54·1 (40·1, 64·8)
Case definition 4	243	79/4953(10·5)	164/4870(22·2)	53·0(38·6, 64·1)

Table 5-2. Efficacy analysis by follow-up time after first-dose vaccination

Follow-up time (after first-dose vaccination)	Total No. of cases	Vaccine	Placebo	Vaccine Efficacy (95%CI)
		n/N(incidence density)	n/N(incidence density per 100 person-year)	
Within 14 days	63	32/6195(11·4)	31/6201(11·0)	-3·3(-4·8, -1·9)
Within 28 days	104	38/6195(5·7)	66/6201(9·8)	42·5(32·9,50·7)
Within 42 days	158	48/6195(8·1)	110/6201(18·5)	56·5(49·6,62·5)
Within 56 days	221	63/6195(7·6)	158/6201(19·1)	60·4(56·5,63·9)
Within 70 days	274	86/6195(8·0)	188/6201(17·7)	54·7(53·2,56·1)
Within 84 days	326	104/6195(8·2)	222/6201(17·7)	53·7(52·7,54·7)
Within 98 days	357	116/6195(8·4)	241/6201(17·6)	52·5(51·9,53·1)
14-28 days after 1 dose*	18	1/5709 (1·3)	17/5697 (21·6)	94·0 (55·1, 99·2)

*For participants who received only single dose vaccination.

Table 5-3. Efficacy analysis by exposure history to SARS-CoV-2

Exposure to SARS-Cov-2 pre-vaccination	Total No. of cases	Vaccine	Placebo	Vaccine Efficacy (95%CI)
		n/N(incidence density)	n/N(incidence density per 100 person-year)	
Unexposed				
Score 2 and above	200	67/3637(13·3)	133/3587(26·8)	50·5(33·6, 63·1)
Score 3 and above	27	2/3637(0·4)	25/3587(4·5)	92·1(66·7, 98·1)
Score 4 and above	10	0/3637(0·0)	10/3587(1·8)	100·0(56·0, 100·0)
Severe	6	0/3637(0·0)	6/3587(1·1)	100·0(16·3, 100·0)
Exposed				
Score 2 and above	9	3/401(5·9)	6/408(11·7)	49·5(-101·8, 87·4)
Score 3 and above	0	0/401(0·0)	0/408(0·0)	NE
Score 4 and above	0	0/401(0·0)	0/408(0·0)	NE
Severe	0	0/401(0·0)	0/408(0·0)	NE

Appendix 6 PROFISCOV Study Group

Instituto Butantan, São Paulo, SP, Brazil

Clinical Trials and Pharmacovigilance Centre

Ricardo Palacios, Mônica Tilli Reis Pessoa Conde, Roberta de Oliveira Piorelli, Elizabeth González Patiño, Hugo Alberto Brango García, Joane do Prado Santos, Rodrigo Piske Finotto, Ana Paula Batista, Camila Santos Nascimento Albuquerque, Flávia Marilia Cestari Magalhães, Carolina de Moura Albino, Rafaela Fernandes Silva, Paloma Bomfim, Luiz Henrique Moraes Caetano de Camargo, Mirian Nascimento

Quality Control Laboratory

Patrícia Dos Santos Carneiro Matheus Trovão de Queiroz, Rubia Galvão Claudio

Development and Innovation Center

Viviane Fongaro Botosso, Soraia Attie Calil Jorge, Fabyano Bruno Leal, Renato Mancini Astray

Scientific Development Center

Sandra Coccuzzo Sampaio Vessoni, Mauricio Cesar Ando, Guilherme Rabelo Coelho, Monique da Rocha Queiroz Lima

University of São Paulo

School of Medicine. São Paulo, SP, Brazil

Clinical Research Center II and Research Medical Laboratory – LIM 60, Department of Infectious and Parasitic Diseases, Clinicas Hospital

Esper Kallás , Amanda Caroline Ribeiro Sales, Amanda Nazareth Lara, Angela Carvalho Freitas, Angela Naomi Atomiya, Bárbara Labella Henriques, Camila Rodrigues, Camila Sunaitis Donini, Danielle Rodrigues Alves, Elizabeth de Faria, Fábio De Rose Ghilardi, Joana Ramos Deheinzelin, Jorge Salomão Moreira, Juliana Ishimine da Silva, Karine Armond Bittencourt de Castro, Leon Capovilla, Livia Zignago Moreira dos Santos, Luara Teófilo Pignati, Luiz Gonzaga Francisco de Assis Barros D'Elia Zanella, Mariana Maria Rocha Santos de Souza, Marília Bordignon Antonio, Marjorie Marini Rapozo, Michel Silvio Duailibi, Natacha regina de Moraes Cerchiari, Patricia Rocha de Figueiredo, Pedro Henrique Fonseca Moreira de Figueiredo, Raphaella Goulart de Souza Vieira, Renata Pissuto Pinheiro, Ricardo de Paula Vasconcelos, Rosário Quiroga Ferrufino, Simone de Barros Tenore, Tatiana Fiscina de Santana, Zelinda Bartolomei Nakagawa, Elaine Cristina Bau, Lilian Ferrari, Denivalda da Silva Gomes Araújo, Gabriela de Castro Keller, Ketlin Kauane Cordeiro Santos, Rosângela Vitória Soares Silva, Beatriz Sales Mourão, Taynan Ferreira Rocha, Carlota Miranda Paredes, Carolina Cardona Siqueira Lobo, Taís Vargas Freire Martins Lúcio, Athos Nascimento Souza, Elenn Soares Ferreira, Gabriel Lopes Borba, Neivaldo Fiorin, Thiago Evaristo Tavares Luzzi, Denise Sales Mourão, Ederson Santo Xavier, Nailson de Jesus Ramos das Virgens, Thiago Antonio do Nascimento, Bruna Samanta da Silva Moreira, Karine Milani da Silva Dias, Leandro Concolato Miranda, Mary Helen Oliveira Morais, Priscilla Almeida Souza, Rayana Silva Paes, Geovanna Guarnier Cardin Farias, Gustavo Coutinho Rezende, Rosimeire Aparecida da Silva Zabotto, Verônica dos Anjos Souza da Silva, Ana Paula da Silva Barros, Clemildes Vieira de Almeida, Gislayne Aparecida de Lima Marcelino, Jéssica Aparecida Soares, Josélia Bezerra dos Santos, Márcia Alves de São Pedro, Maria Esmelindra Monteiro de Moraes, Helena Tomoko Iwashita Tomiyama, Alberto Hiroyuki Tomiyama, Aline Tatiane Lumertz dos Anjos, Andrea Niquirilo, Claudia Satiko Tomiyama, Elisabeth Alves Pereira, Eric Silvestre, Maria Angelica Alcalá Neves, Raissa Reis Silva, Yasmine Perez Levy Ribeiro, Maria Cândida de Souza Dantas, Issler Moraes da Silva,Renan Fernandes Carvalho

Institute of Tropical Medicine (IMT-SP)

Ester Cerdeira Sabino, Maria Cássia Mendes Correa, Anderson de Paula, Tania Regina Tozetto Mendoza, Mariana Severo, Jaqueline Goes de Jesus, Flávia Sales, Erika Manuli, Darlan da Silva Cândido, Ingra Morales

Laboratory of Clinical and Molecular Virology, Department of Microbiology, Institute of Biomedical Science. São Paulo, SP, Brazil

Edison Luiz Durigon, Danielle Bruna Leal de Oliveira, Erika Donizette Candido, Guilherme Pereira Scagion

Department of Internal Medicine, Ribeirão Preto Medical School, Ribeirão Preto, SP, Brazil

Eduardo Barbosa Coelho, Silvia Caroline Santana Moura Carvalho, Bárbara Cristina Santana Mello, Fábio André Dias, Marina Silva Campos, Juliana Rezende, Marilda Aparecida Ribas, Soraya Regina Abu Jamra, Daniela Aparecida Lorencini, Simone Aparecida Mattos Garcia, Onildo Passafaro, Ariane Teixeira Vicente, Diogo Henrique Martins, Ana Carolina Conchon Costa, Fabiana Alves Rodrigues Druzili, Carolina Leite Freitas, Luciana Mara Cangemi, Aila Mabla Azarias de Castro, Diego Lorencini, Leila Cristina Franco dos Santos, Janaina de Andrade Pereira, Taiz Francine Brasil da Silva, Ramaiane Aparecida Pugnolli, Ana Rayza Palaretti, Ana Claudia Aparecida Rodrigues, Camila Pereira, Jessica Merighe Godoi, José Fernando Aguiar, Nathalia Ziegler Oliveira, Ana Carolina Andrade Emerenciano, Barbara Marques Coutinho, Andrea Moreira de Freitas, Fernanda Munari Meneguim, Bianca Rizzotto Ferreira, Luan Lucas Reis da Costa, Gabriel Bazo, Fabio da Veiga Ued, Tatiane de Paula Rosa, Daiana Cristina Lorencini Aguiar, Gabriela Gimenes Faustino Ilana, Amanda Hereman Malavasi, Mayara Gomes Brito, Lucas Braz Barbosa Coelho

Emilio Ribas Institute of Infectious Diseases, São Paulo, SP, Brazil

Luiz Carlos Pereira Junior, Ana Paula Rocha Veiga, Guilherme Assis dos Anjos, Alexandre de Almeida, Najara Ataide de Lima Nascimento, Diogenes Coelho Junior, Gabriela Prandi Caetano, Ana Carla Carvalho de Mello e Silva, Rafael Affini Martins, Natália Mercedes Cabral Amdi, Cinthya Mayumi Ozawa, Magna Magalhaes Siva, Anna Karina Queiroz Mostachio, Katia Sayama Tsutui, Ana Paula Augusto dos Santos, Alessandra Moreti dos Santos, Marcia Aparecida dos Santos Gouveia, Lilian Mathias Moreira, Léia Dias Barbosa, Margarete Rodrigues de Carvalho, Mirian Ishi, Mariana Takahashi Ferreira Costa, Nelson Alberto Freitas Guanez, Odijoselia Ferreira de Sá, Virginia Barbosa Leite, Luciana Elizabete Cunha, Ivan Máximo da Silva, Vinicius Silva Araújo, Marian Romero Soares Rodrigues, Adriano Samoel Batista de Souza Nascimento, Marco Aurélio Conceição, Catia Dionisio dos Santos, Luciana Aparecida Pereira, Alessandra de Fatima Margarida, Debora Carla dos Santos, Andréia Aparecida Hermann, Ana Kesia de Souza Lima, Nailda Dantas Nunes Leal, Sergio Luiz de Lara Campos, Mariane Pereira, Caroline Franco Zanotti, Paloma Martins Vieira, Paloma Priscila Rocha Aronca, Denis Jose Fumagali, Alpetras Martins Maciel, Thais Monteiro Nunes Pereira, Milton Tadeu da Silva, Bruno Tenório de Oliveira, Maria Elena Ana Correa da Silva, Margarete Leme, Mercia Rocha Moreira,

Silvia Garcia, Vlaudeflide dos Santos, Maria Elizabete Mendes Alves dos Santos, Bianca Carolina Ferreira, Sonia Silva Ferreira, Leticia Kadiri da Silva, Neide Aparecida Leite dos Santos, Fernanda Lima Arruda, Vilma Aparecida Adami, Claudia Solange da Silva

Faculdade de Medicina de São José do Rio Preto (FAMERP), São José de Rio Preto, SP, Brazil

Mauricio Lacerda Nogueira, Cassia Fernanda Estofolete, Karen Sanmartin Rogovsky, Samuel Noah Scamardi, Camilo Alberto Correa de Vasconcellos Dias, Altaís Helena Camargos Robles, Ingrid Emily Alencar Bento, Bruna Basaglia, Elis Regina da Silva Ferreira, Danatielle Mega Ferreira, Eliane Aparecida Fávaro Pereira, Ana Maria Dias de Sousa Marconi, Talita Garcia Lopes Viçoso, Bethania Cirqueira de Oliveira, Hellen Carla Padovani, Theo Rodrigues da Silva, Elissandra Josepha Eugenia Machado Batista, Francielli Regini Carvalho de Faria, Carla Gabriela de Lima Mattos, Kamilla Amaral David Rocha, Rafael Alves da Silva, Bárbara Boreli Almeida, Marini Lino Brancini, Solange de Fátima Vargas Scaranaro, Marilia Mazzi Moraes, Lorena Fernanda da Silva, Debora Rodrigues de Brito, Victor Miranda Hernandes, Sonia Aparecida Miranda, Debora Rodrigues de Brito, Andresa Lopes dos Santos, Henrique Munhoz Moya Gimenes, Silvio Orlando Isso, Perpetua Pereira Dias Fernandes, Eduarda Veronezi Claro, Raphaely Rodrigues Obara, Erica Seixas Floriano, Gislaine Celestino Dutra da Silva

School of Medical Science and Hospital of Clinics, State University of Campinas – UNICAMP, Campinas, SP, Brazil

Francisco Hideo Aoki, Mariangela Resende, Adriane Maira Delicio Abati, Paulo Afonso Martins Abati, Diego Cassola Pronunciato, Alisson Aliel Vigano Pugliesi, Letícia Pisoni Zanaga, Eduardo Hiroshi Tikazawa, Marcelo Gustavo Lopes, Giovana Cury Queiroz, Anna Kim, Maria Helena Pavan, Alex Bento de Carvalho, Ehideé Isabel Gómez La-Rotta, Fernanda Sucasas Frison, Edite Kazue Taninaga, Inajara de Cassia Guerreiro, Leila Tassia Pagamicce, Cleusa Gimenes dos Santos, Sofia Domingues Barthman, Maria Silvia Kroll, Elisangela Aparecida Ramos Domingos, Rosilene Balbina de Souza, Liene Gomes Magossi, Hamilton Bertan, Lara Paro Dias, Mayra Carvalho Ribeiro, Mariane Galvão Roberto Tavares, Mariana Vieira Morau, Rafael Nishimoto, João Kleber Novais Pereira, Regina Helena Dahas de Carvalho, Kely Carolyne Alves de Sousa, Nanci Michele Saita, Maria Valéria de Omena AThyde, Cristina Medeiros da Silva Aguilar, Valéria Galdino, Robson Pereira da Silva, Luisa Lazarini Rubio, Elisandra Valéria Negrison Calixto, Manoel Vicente Carminitti Feiteiro, Vagner Oliveira Duarte, Ronald Jorge Menghini dos Santos, Rosangela Aparecida dos Santos, Eliana Ferreira Paes, Ingrid Rocha Franco de Lima, Edilene Menezes Sabino, Marcos Roberto Guimarães, Karina Dias Teixeira Vieira, Diogo Rodrigues Machado, Maxlei Silveira Marini, Graciela Amaral, Fabio Augusto

Cavaglieri Feiteiro, Elaine Cristina Paixão de Oliveira, Talita Lobato de Souza, Vanessa Alcântara de Carvalho, Edjane de Oliveira Marinho Feiteiro, Victor Rodrigues Olivato, Letícia Pinheiro de Mattos, Francisca Pontes Santinoni, Jessica do Nascimento, Mariana de Campos, Natalie de Oliveira Alves

Universidade Municipal de São Caetano do Sul, São Caetano do Sul, SP, Brazil

Fabio Eudes Leal, Aline Lopes de Almeida, Maria Laura Mariano de Matos, Letícia Cleto Duarte Sugiyama, Gabriela Mora Santos, Cristiane Alves da Silva Ferraz, Caroline Machado Nunes, Leticia Reis Lopes, Amarilys Luiza Druziani, Marine Antunes Pires, Gabriela Júlio Fernandes Viana, Fernanda de Almeida Euclides, Sônia Yoo Im, Suzane Pereira da Silva, Claudia Cristina Ferreira Ramos, Carolina de Oliveira Silva, Giovana Ferreira Pinheiro, Isabelle Moiano Roseira Galo, Carina Vitória Paradas Dias, Livia Mirella Mengar, Nicole Gallone Rizzo, Renata Nunes Achar Fuji, Andrea de Barros Coscelli Ferraz, Michelle Cirilo, Andreia Inacio Avelino, Vanessa Vieira Hornink, Cristina Antonia de Jesus Catalã, Selma Arnold da Silva, Erika Regina Manuli, Milena Borges, Eric Boragan Gugliano, Milena Pereira Canavesi Caetano, Yara Chinaglia, Fernando Monteiro de Sá Luiz, Raquel da Silva Terezam, Giselia Sabino Cruz, Luma Ramirez de Carvalho, Ranilson José da Silva, Edvaldo Alves, Giovana Lourenço Munhoz, Luiza Caroline Rinaldi, Carla Cristina Munhoz, Maria José Barzan, Stella Maris Pereira Sobrinho Rodrigues, Bárbara Suéllen Guimarães Marin Ferreira, Tainaira da Silva Gramalio, Carla Andrea Ciarineli, Bruna Fornazieri Piotto, Kimberley Yaunde da Silva Matos

Instituto Israelita de Ensino e Pesquisa Albert Einstein, São Paulo, SP, Brazil

Luis Fernando Aranha Camargo, Carolina Devite Bittante, Telma Priscila Lovizio Raduan, Mariana Silva Soares, Maria Clara Pimentel Lopes, João roberto Resende Fernandes, Kamilla Ferreira de Moraes, Ariane Teixeira Vicente, Ericka Constantinov Oliveira, Aleksandra Cristina Mussi, Cristiane Okada, Bruna Camargo Gonçalves, Roberta Carolina Haddad, Caroline dos Reis Pedroso, Mariza Kogake Nocamatsu, Elke Ferreira Salim, Tarsila Gomes Feijão de Oliveira, Elaine de Jesus Santos, Bruna Camargo Gonçalves, Daniela Regina Gusmão Ferreira, Luciane dos Santos Vieira Alves, Hannah Maureen Garcia Mota, Mariana Gabriela Rodrigues, Gisele Alves Pinheiro, Luiza Blumer Ribeiro, Isabel Cristina dos Santos, Regiane Mendes Brandão, Ariana Silva de Lima Teixeira, José Carlos da Silva, Rosana Soares Gomes, Fernanda Oliveira Marcelino da Silva, Giselia Martins Dantas Silva, Aparecida Sampaio Sousa, July do Nascimento Alves, Thais Helena Costa Petrin, Daniela

Boschetti, Amanda Tarratacada Guimarães, Danilo Souza Vanglerini, Juliana Alves de Lima, Letícia de Mello Soares, Julia Alves de Lima, Adriana Guilherme, Lorena Silva Brasil, Felipe Rodrigues Nogueira Silva, Diana Freire de Brito, Rebeca Cezário Freire de Araújo, Sandra Rodrigues Rocha, Lucas Soares Cardoso dos Santos, Raquel Fidelis Teixeira Bonfim da Silva, Jeane Silva Menezes, Talita Santos Martins Vaglerini, Danielle Santana de Oliveira

Research Institute of Cancer Hospital, Hospital de Amor, Barretos, SP, Brazil

Gecilmara Cristina Salviato Pileggi, Paulo Tarso Oliveira, Raquel Aparecida Reis Teixeira, Luis Guilherme de Freitas Rodrigues, Guilherme Corraleiro Martins, Maria Luisa Corcoll Spina, Laila Genoefa Bortot, Renan Cesar Zanon Teixeira, Isabela Silva Pasqua, Janaina Zambon de Oliveira, Juliana Doblas Massaro, Julia Anelli Roncador, Karla de Picoli Alexandre, Daniel Aquilino Oliveira, Tais Vaz, Izabella da Silva Oliveira, Nathalia Martines Tunissiolli, Silvania Rodrigues dos Santos, Cristiane Lima do Carmo, Maicon Fernando Zanon da Silva, Felipe Alexandre Machado, João José Mandu Confetti, Fernanda Amâncio da Silva Giroli, Isabela Haddad Peron, Carla Roberta M. B. de Sousa, Joviany Talita da Silva, Camila Rodrigues Marques Fornazier, Stefani Regina da Costa Lopes, Aline Larissa Virginio da Silva, Beatriz da Silva Ventura, Layane Cristina dos Santos Vaz, Thiago Jose Donizete Simão, Thais Kapp Gonçalves

Centro de Pesquisa e Desenvolvimento de Fármacos, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

Mauro Martins Teixeira, Astaruth Guimarães Froede, Alexandre Moraes Periard, Tallyne Tayna Barros Lana, Marina Neves Zerbini de Faria, Último Libânio da Costa, Carolina Morais Milan de Oliveira, Gabriela Carvalho Abreu, Erickson Ferreira Gontijo, Lisia Maria Esper, Angela Vieira Serufo, Fátima Maria Caldeira Brant Costa, Josiane Pinto Moreira Vaz, Rosângela Santos Pereira, Vitor Magno Cardoso Rocha, Rafael Leite Ribeiro dos Santos, Tarciana Batista Teixeira, Marlene do Rozario Silva, Miriam Siqueira Araújo, Luana Patrícia Gonçalves Silva, Josiane Lino dos Santos Frattari, Tatiana Dias Calábria Santos, Thais de Menezes Noronha, Alessandro Ferreira de Macedo, Ana Rosa Rodrigues Silva, Daniela Cristina de Oliveira Pontes, Jucelina Gonçalves de Oliveira, Lorena Augusta Coelho de Mattos, Priscila Sena Lopes, Maria Cecília da Silva Ferreira, Tânia Mara Gomes de Pinho, Amanda Lino dos Santos, Diego Wagner Tarquino, Luciana Mara Costa Moreira, Bruno Vinicius Santos Valiate, Raquel Patrocinio Simoes, Letícia Soldati da Silva, Déborah Caroline Salgado Stoupa, Phillip Diego Carajá, Ilma Marçal de

Souza, Patricia Candida Messias Santana, Lorrayne Madaline Costa Tarquinio, Renata de Jesus Oliveira, Eva Eunice de Fátima Nascimento, Girlane Santos de Oliveira, Amanda Mara Gomes de Pinho, Gislene Moreira

Department of Infectious Diseases, Hospital de Clínicas, Universidade Federal do Paraná, Curitiba, PR, Brazil

Sonia Mara Raboni, Cristiane Secco Rosário, Débora Carla Chong e Silva, Giovanni Luis Breda, Andrea Maciel de Oliveira Rossoni, Maria Cristina Vilatore Assef, Susane Edinger Pereira, Tony Tannous Tahan, Betina Mendez Alcântara Gabardo, Carlos Antônio Riedi, Cleverson Alex Leitão, Débora Silva Carmo, Fabricio Salles Rosa Solak, Juliana Mayumi Kamimura Murata, Leniza Costa Lima Lichtvan, Tyane de Almeida Pinto Jardim, Tiago Hessel Tormen, Elessandra Souza Bitencourt, Maria Gabriela Mendes Pereira da Costa, Aline de Fátima Bonetti, Joelize Claudiane Stanoga Denk, Gisele Weissheimer, Luciana Michele Mello, Natália Fracaro Lombardi Asinelli, Ana Beatriz Guedes Ribeiro, Antônio Eduardo Matoso Mendes, Juliane Carlotto, Mayara Caroline Barbieri, Simone Gomes de Souza, Tânia Maria Araújo, Vivian Carnier Jorge, Inajara Rotta, Gisele de Paula e Silva Carneiro Mendes de Souza, Indianara Rotta, Irene de Morais, Klezia Morais da Silva Belletti, Juracy Anisio da Silva Neta, Valquiria Daniele Casanova Antunes, Sabrina Karim Mota Ribeiro, Meuryely Euleny Macedo da Silva, Alice dos Santos Almeida, Patricia Aline dos Santos Pançolin, Ester Silvino da Costa Paris, Larissa Izabelle Machado, Genilberta de Meireles Biscarde, Jean Pierre Guinapo Franco Marques, Jennifer de Souza Anhaia, Maria Aparecida Pereira da Silva, Rosangela Aparecida de Almeida de Fitz, Simone Albertoni Souza, Simone Pereira Leal, Tiago Rodrigues de Souza, Vilmara Aparecida Salles Machado, Patricia Proença Santana, Atila Brizola Ribas, Bárbara Kathleen Gonçalves Poletto, Alexandre Paim Mendes, Daniel Barros Teixeira Leite, Eduarda Sampaio Lazzarotto, Erika Leticia da Rocha, Ione da Silva Santos Mocelin, Jaqueline Fiamoncini, Guilherme Paim Mendes, Larissa de Souza Ferreira, Maria Regina de Mattos, Liriane Aparecida Pedroso Vaz, Mariana Likoski Pereira, Nildson Eduardo Pinheiro Souto Junior, Lucienne Dronneau, Rosangela Salete Ribeiro dos Santos, Rosangela de Fátima Prestes, Vinicius Lemos Brito, Vilmara Heimoski Teixeira, Thays Fischer, Laura Holtman Ferreira, Suzana Beatriz Borsato Crissi, Andressa Zabudowski Schroeder, Leonardo Filipetto Ferrari, Guilherme Stapasolla Vargas Garcia, Barbara Kawano Raposo, Gustavo Osmarin Tosti, José Marcio Camargo Junior, Meire de Souza Vieira, Letícia Mari Tashima, Carolina Ayumi Ichi, Micaela Hedeke Paim Mendes, Isadora Heimoski Escobar, Alberto Memari Pavanelli, Daniel Balaban, Fernanda Panacioni, Isabella Chapieski, Luana Francine Anad, Eduardo Mendonça Soares, Luiza Moschetta Zimmermann, Isabela Cristine Torres, Jessica Castro da Silva

Infectious Diseases Service, Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil.

Fabiano Ramos, Clarissa Tabajara Moura, Ingridy Cortes da Silveira, Cindy Johan Jara Hernandez, Hilda Janneth Monroy Hernandez, Jae En Chung, Daniel Fernando Arias Betancur, Luciana Zani Viegas da Silva, Ana Carolina Müller Beheregaray, Gabriel Bottin Marques, Débora Wilke Franco, Amabile Ribeiro de Oliveira, João Pedro Volkmann Amaral, Michelle Stump Viegas, Gabriel Azambuja Athaydes, Deise do Nascimento de Freitas, Andressa de Oliveira, Leydy Yhoana Quevedo Diaz, Leonardo Ferraz de Bittencourt, Hernando Salles Rosa, Laura Trevisan, Isabelli Guasso, Daiane Monteiro Marques, Kelli Regina Brand dos Santos, Alice de Lima Alves, Caroline Cavalheiro Biazussi, Thays Maiato Pereira, Aline Nunes Leal, Roseli Andreia Borges da Costa, Allan Pereira, Rafaella Ruiz do Couto, Luiz Airton Rocha de Freitas, Bruna Isabel Silveira Costa, Larissa Rafaela de Lima Sanches, Fernanda Paula Polaczinski Pich, Mariana Horn Scherer, Thamiris Carvalho Vargas, Mariana Horn Scherer, Rodrigo Volf dos Santos, Rodrigo Flores Fraga, Tasiana Aylen Cervellera Simonetti, Otávio Freitas de Moraes Ogrizek,Michelle dos Santos Leite, Janaina Moraes de Araujo, Carine Padilha de Oliveira, Karla Durante, Renata de Araujo Montechiaro, Elisandra Simonetti Cervellera, Bianca Luisa Rodrigues Lopes, Krislayne da Silva Rosa

Internal Medicine Department. School of Medicine, Universidade Federal de Pelotas. Pelotas, RS, Brazil

Danise Senna Oliveira, Adrienne Sassi de Oliveira, Bruna Gazoni de Souza, Carolina Avila Vianna, Daniel Brito de Araujo, Paulo Orlando Alves Monteiro, Susane Müller Klug Passos, Luciane Maria Alves Monteiro, Clara Camacho dos Reis, Eduardo Coelho Machado, Carla Vandame da Silva, Cristofer Magro, Lorena Zaine Matos Martinho, Caren Laís Seehaber Friedrich dos Santos, Giulya da Silva Ribeiro, Rodrigo de Almeida Vaucher, Bianca Machado de Ávila, Luana Bonow Wachholz, Bruna Quintana Nizoli, Fernanda Severo Sabedra Sousa, Maura Geovana Farias Soares, Aline Machado Carvalho, Daniela Nunes Schaun de Siqueira, Leandro Rodrigues de Rodrigues, Fabiana Kommling Seixas, Thaís Larré Oliveira, Bruna Silveira Pacheco, Tiago Vieiras Collares, William Borges Domingues, Adriane Geppert, Samuel Silva dos Santos, Marcia Bandeira da Luz Baladão, Charles Ramalho Ioost, Gilda Nara Oliveira Barros da Silva Marcos Dinael Klug Stifft, Tatiane Jacobsen Rodrigues, Tatiana Vieira Macedo, Camila Bonemann Bender, Mariana Gállio Fronza, Marcelo de Lima, Vanessa Gali, Isadora André Rosa Lopes, Paola Oteiro de Faria, Mariana de Oliveira Martins, Marcela Bihalva da Silva, Rita Oliveira dos Santos

Center for Tropical Medicine, School of Medicine, University of Brasilia, Brasília, DF, Brazil

Gustavo Adolfo Sierra Romero, Edison Tostes Farias, Alexandre Anderson de Sousa Munhoz Soares, Veronica Cristina de Melo Rocha, Renata Maria de Castro Martins, Rafael Gobbato Brandão Cavalcanti, Camila Rodrigues Ribeiro, Amanda Moreira Parente, Larissa Fernanda Santos Silva, Eduarda Jacinto Bauer, Isabela Roberta Chaves Nunes, Marcia Andrea Seibert Campara, Elizabete Cristina Iseke Bispo, Gyselle Alanna Mota, Larissa Santos Soares, Viviane da Silva Machado, Barbara Manuella Cardoso Sodré Alves, Clarisse de Mendonça Brito, Matheus Eça de Oliveira Felipe, Jacqueline Pereira Oliveira, Patricia Matias Pinheiro, Torlane Renne Dias Rodrigues, Naiara Daris dos Santos, Luciana Franca de Meneses, Fernando de Oliveira Garcia, Cleuza Feliciana de Oliveira, Renata Santos Souza de Amorim, Claudio Lopes Valentim, Lidiane da Silva Queiroz, Sandra Ribeiro Barbosa, Joyce Martins Xavier, Talita Barreto Figueredo, Fabiana Canavieira Araújo, Kaline de Oliveira Medeiros, Leidiane Aparecida Torres da Silva, Suzana Cardoso Mesquita, Patricia Pereira Pires de Sousa, Fernanda dos Santos Lopes, Alceu Sluzala, Camila Ferreira de Moura, Gabrielle Kefrem Alves Gomes, Sandra Maria Ferreira, Adriana Pereira Campos, Marcia Veloso Machado de Mendonça, Roberta Gonçalves da Silva, Juliette Cardoso de Santana, Luciene Valeria Salgado Costa Vasconcelos, Carolina Liane de Macedo Eloi, Jaqueline Martins Xavier

Department of Internal Medicine and Infectious Diseases, Julio Müller School Hospital, Federal University of Mato Grosso, Cuiaba, MT, Brazil

Cor Jesus Fernandes Fontes, Tiago Rodrigues Viana, Rafael Estevanovich Bertoldi Torres, Larissa Botelho Pedrini, William Kleyton de Melo Aguiar, Willian Benedito de Proença Junior, Caroline Reyes, Iury Jocemar Alves, Grasiela Panchoni Menolli, Thiago Ribeiro Nunes Domingues, Pedro de Carvalho Ferreira, Laressa Pereira Sari,,Fábio José da Silva, Gisiene Silva da Luz Moreira, Renata Ito de Araújo, Fabio Alexandre Leal dos Santos, Ruberlei Godinho de Oliveira, Alessandra Emanuelle Cunha Rodrigues, Edna Maria dos Santos, Rosane Christine Hahn, Antônio Gonçalves de Araújo Junior, Maria Luiza Ortiz Nunes da Cunha, Kleythiane Regina Oliveira Costa, Edvania Oliveira Vasconcelos, Juliana Lelis de Almeida, Maria Aparecida Cordeiro de Jesus, Laura de Sousa Dias, Nágisla Maria Benta de Miranda Monteiro, Marly Lopes Soares Monteiro, Ana Lucia Maria Ribeiro,,Jakeline Oliveira da Silva, Rosana Jesuina da Silva, Armando José Guevara Patino, Thaís Maciel Carlos, Palloma Stefany Silva Café, Raimundo Jose da Silva,,Cristina Alves Pereira dos Reis, Igor Gonçalves Baicere,,Fábio Assis de Campos Junior, Emanuelle Soares Camolesi, Pedro Augusto Uecker Paixão,Camila Neves Silva, Hélio Carlos Rocha de Carvalho Junior,Welliton Batista de Morais, Laís Maria de Assis e Silva

Hospital Universitário Maria Aparecida Pedrossian, Universidade Federal de Mato Grosso do Sul

Ana Lúcia Lyrio de Oliveira, Rivaldo Venâncio da Cunha, Déborah Lemes Nogueira, Iêda Maria Rodrigues Vilela Demirdjian, Keila Ventura Soares, Leonardo Martinez Lourenço de Oliveira, Matheus Baptista Passos, Michaela de Oliveira Tognni, Susan Gomez Chambi, Izilyanne Lucas Hoscher Romanholi, Fernanda Paes Reis, Izadora Bonfim, Lethicia Farias Marcino, Lívia Alves da Silva, Thays Cristina Ferreira Ramos, Wagner de Souza Fernandes, Alessandra Moura da Silva, Cristiane de Sena Silva,Ione Maria Lobo dos Santos, Luiz José Gonçalves,Marcos Geronimo da Silva, Maria Aparecida dos Santos Eloy,Carlos Alberto Martins da Rocha, Naiara Valera Versage, Patricia Matos Coelho Vila Real, Rafael Ovando Fraiha, Debora Martins Cardoso, Jaciara de Souza Correa, Helena Pereira Vargas, Marcos Antonio Sebastião, Suelen Kelee Amarilha Gimenes

Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

André Machado de Siqueira, Ellen Fraga Won Randow Falcão, Carolina Pereira Carvalho Lucas, Christiane Fialho Gonsalves, Guilherme Téo Pinto Santoro, Tatiana Fukui da Silveira, Marcelle Alaluna Freitas e Silva, Laura Santos Oliveira, Bianca Silveira Moreira, Eliesier da Silva Souza Filho, Marina Barreto Alvarenga, Claudia Roberto da Silva, Camila Rosas Neves, Thainá do Couto Nabarro Fraga, Camila Arantes Ferreira Brecht D'Oliveira, Lua Kaihe Varjão de Oliveira Cerqueira, ,Caroline Loureiro Hildebrant,,Fabrício da Silva Rangoni, Darlizy Rodrigues Santos, Katia Lucia da Silva Scherz, Karine Carrilho Santos, Vanessa da Silva Porto, Tábatha de Souza Vasconcelos, Cíntia Ferreira Gonçalves, Daniele dos Santos Silva Fernandes, Christiane Berger, Francielle Mayer Guimarães, Marcos Vinícius Souza de Almeida, Katia Regina de Oliveira Azevedo Rocha, Thays Basilio Oliveira, Ana Carolina Almeida Fortes, Katharina Ferreira Araújo, Barbara de Azevedo Scangarelli, Ana Carolina Castro de Jesus

Sinovac Biotech Co., Ltd, Beijing, People's Republic of China

Gang Zeng, Qianqian Xin, Weining Meng

1.18. Vacina do Butantan tem eficácia global superior à exigida pela OMS

O Instituto Butantan e o Governo de São Paulo informam que a vacina contra o coronavírus obteve 50,38% de eficácia global no estudo clínico desenvolvido no Brasil, além de proteção de 78% em casos leves e 100% contra casos moderados e graves da COVID-19. Todos os índices são superiores ao patamar de 50% exigido pela OMS (Organização Mundial de Saúde).

Os resultados foram submetidos a um comitê internacional independente e já estão com a Anvisa (Agência Nacional de Vigilância Sanitária), que analisa o pedido de uso emergencial do imunizante no Brasil. A pesquisa envolveu 16 centros de pesquisa científica em sete estados e o Distrito Federal. O teste duplo cego, com aplicação da vacina em 50% dos voluntários e de placebo nos demais, envolveu 12,5 mil profissionais de saúde.

"É uma excelente vacina esperando para ser usada em um país onde morrem, no momento, em torno de mil pessoas por dia. Esperamos que as autoridades entendam o momento e ajudem nossa população a receber as vacinas o mais rapidamente possível", afirmou o Diretor do Instituto Butantan, Dimas Covas.

"Os dados são extremamente importantes no impacto da saúde pública, impedindo que as pessoas adoeçam de forma grave e sobrecarreguem hospitais. É a possibilidade de impedirmos que as pessoas morram", disse o Secretário de Estado da Saúde, Jean Gorinchteyn. "Temos uma vacina que foi testada na vida real, no meio de uma pandemia e naqueles que eram mais expostos", acrescentou.

O estudo verificou que a menor taxa foi registrada em casos de infecções muito leves, considerados score 2 e verificados em pacientes que receberam placebo. De uma amostragem de 9,2 mil participantes, 85 dos casos muito leves foram de pessoas que receberam vacina, e 167 em voluntários que tomaram placebo.

Já o resultado de eficácia dos casos leves, classificado como score 3, em pacientes que

precisaram receber alguma assistência, foi de 77,96%, sendo que sete pessoas haviam recebido a vacina, e outras 31, placebo.

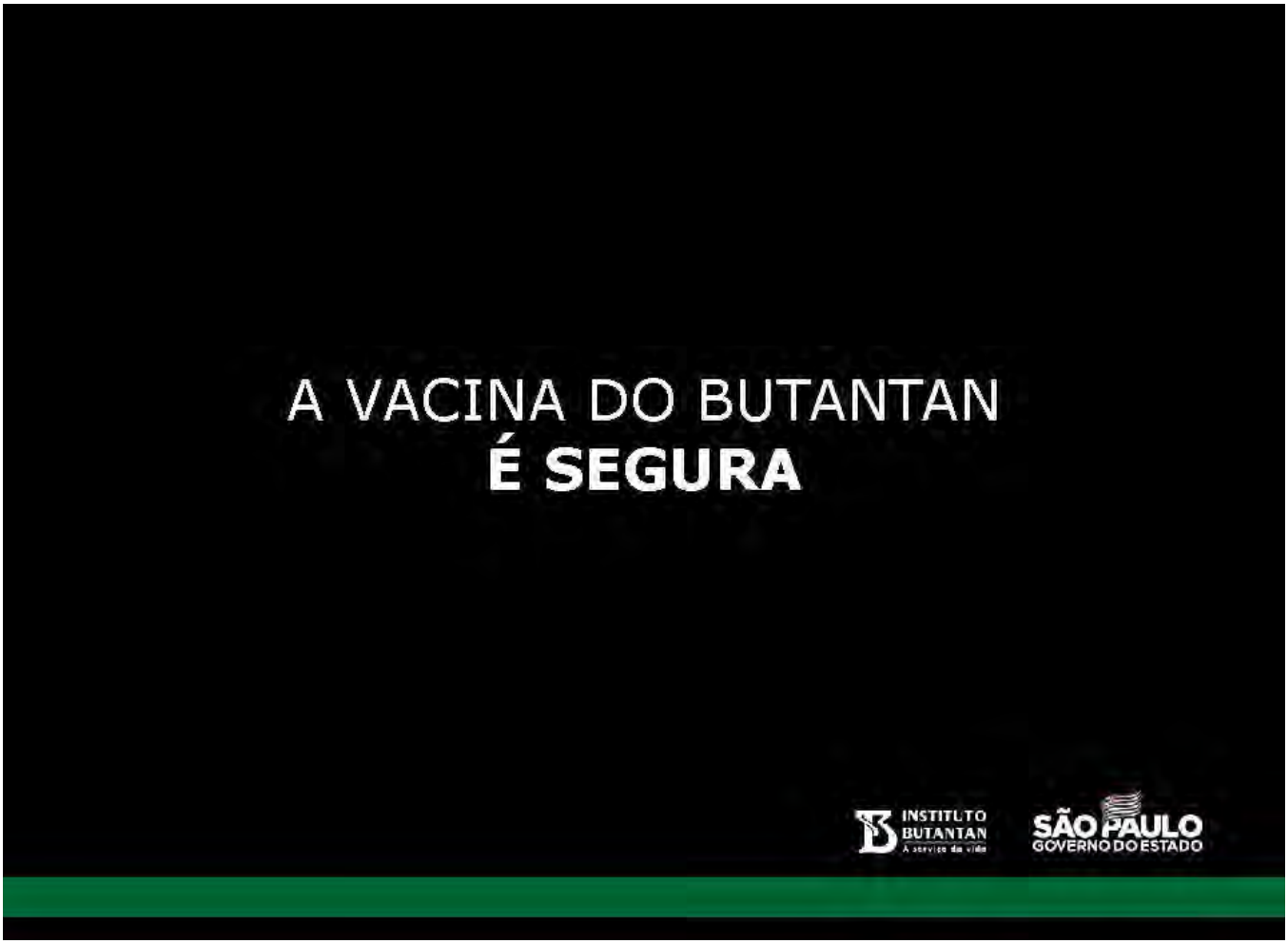
Para os casos moderados e graves que necessitaram de hospitalização, a eficácia foi de 100%. Nenhum paciente infectado que recebeu a vacina do Butantan precisou de internação. Entre os que tomaram placebo, houve sete pacientes que precisaram de internação. Todo os voluntários são profissionais de saúde, com risco muito alto e contínuo de exposição ao coronavírus. Eles receberam duas doses da vacina, com intervalos de duas semanas entre cada aplicação. A pesquisa também demonstrou que o imunizante é extremamente seguro - nenhuma reação adversa grave foi registrada entre os participantes.

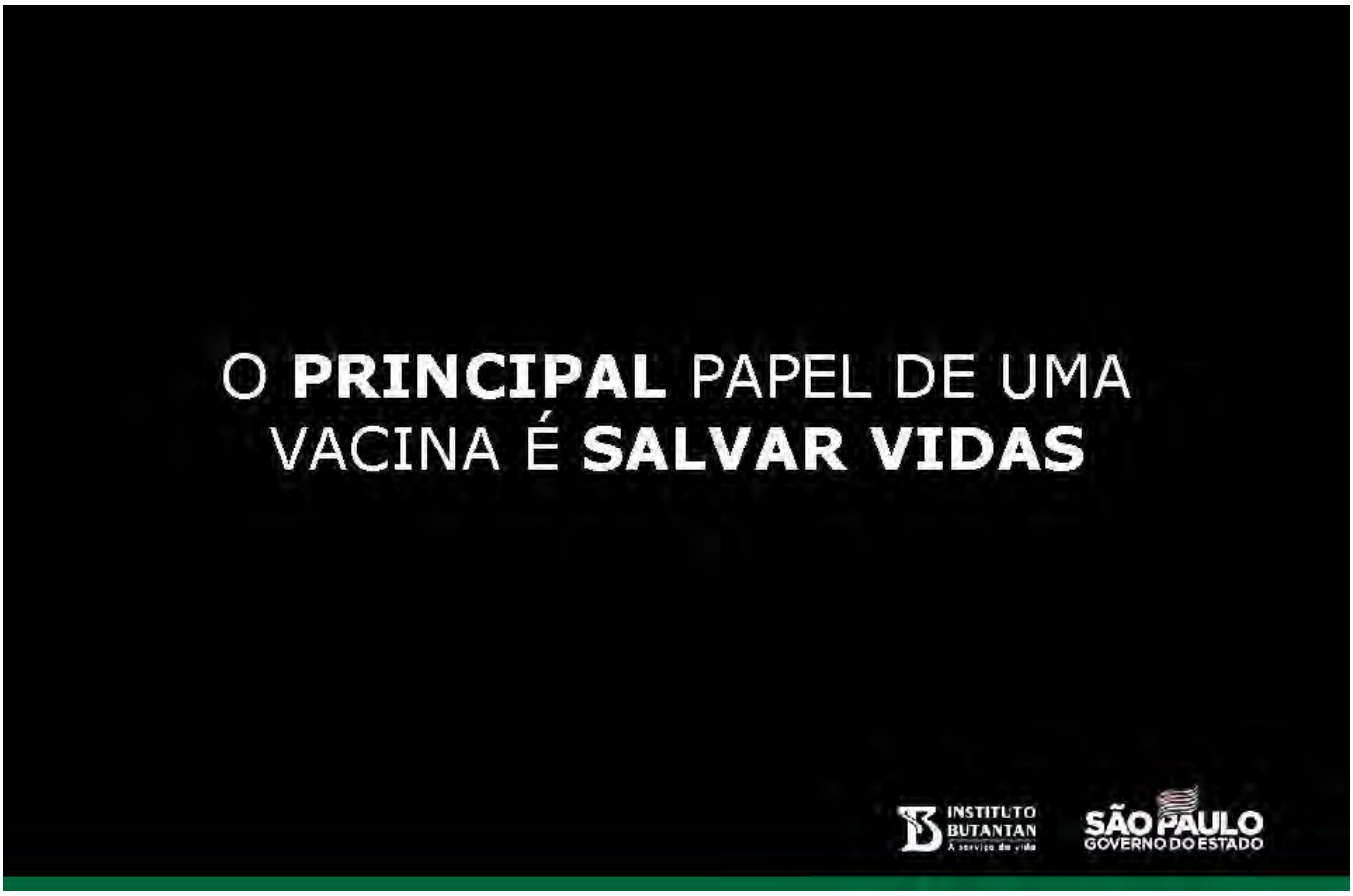
A vacina é desenvolvida pelo Butantan em parceria internacional com a biofarmacêutica Sinovac Biotech, sediada em Pequim. O produto é baseado na inativação do vírus SARS-CoV-2 para induzir o sistema imunológico humano a reagir contra o agente causador da Covid-19. A tecnologia é similar à de outras vacinas amplamente produzidas pelo instituto de São Paulo.

Em novembro de 2020, a revista científica Lancet, uma das mais importantes no mundo, publicou os resultados de segurança da vacina do Butantan nas fases 1 e 2, realizados na China, com 744 voluntários. A publicação mostrou que o produto é seguro e capaz de produzir resposta imune em 97% dos casos em até 28 dias após a aplicação.

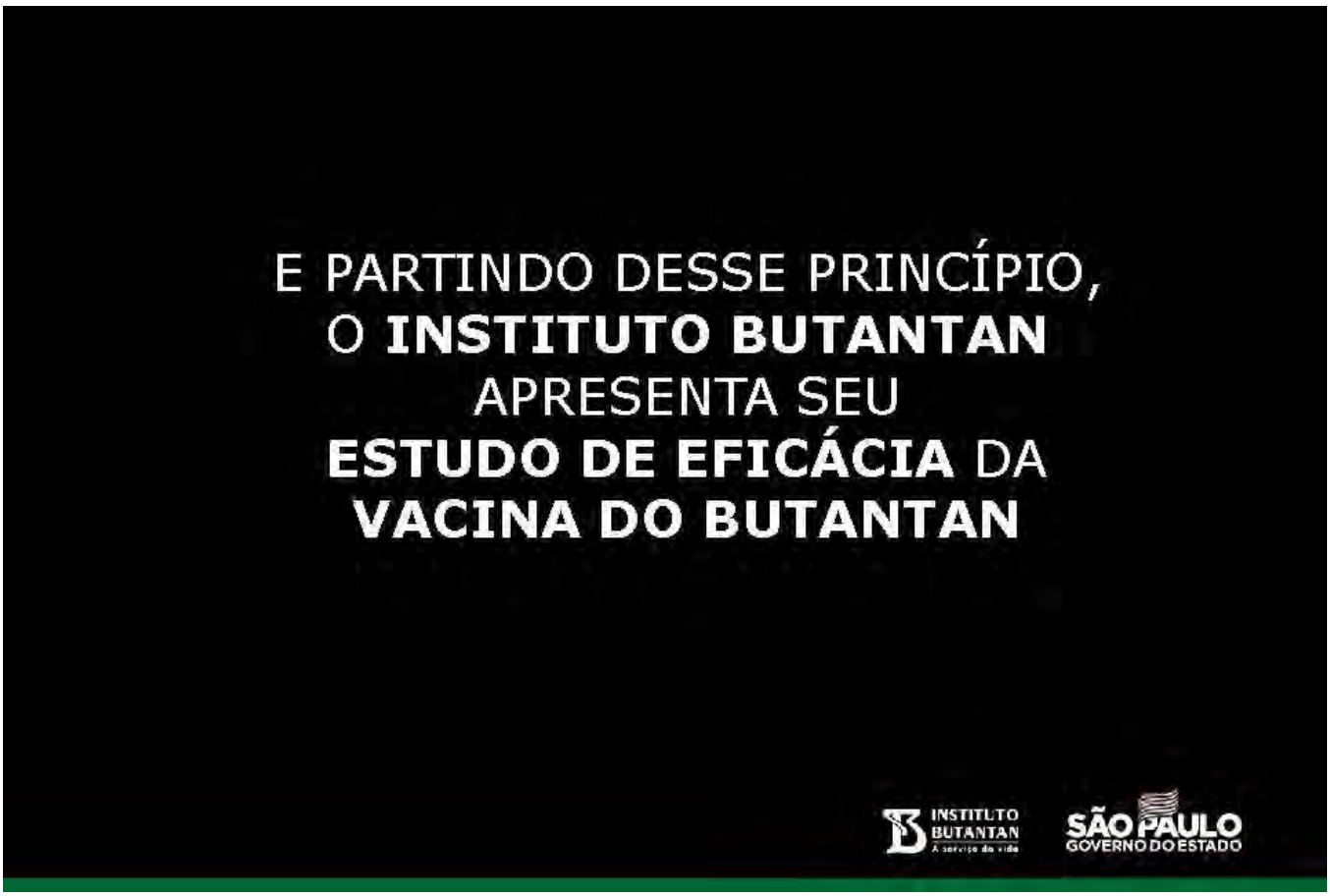
Publicado em: 14/01/2021


SÃO PAULO
GOVERNO DO ESTADO
Vacina do Butantan
A vacina do Brasil
2021


A VACINA DO BUTANTAN
É SEGURA
INSTITUTO BUTANTAN
SÃO PAULO
GOVERNO DO ESTADO


O **PRINCIPAL** PAPEL DE UMA VACINA É **SALVAR VIDAS**
INSTITUTO BUTANTAN
SÃO PAULO GOVERNO DO ESTADO


OS GRUPOS DE **MAIOR RISCO** SÃO SEMPRE **PRIORIDADES**
INSTITUTO BUTANTAN
SÃO PAULO GOVERNO DO ESTADO


E PARTINDO DESSE PRINCÍPIO,
O INSTITUTO BUTANTAN
APRESENTA SEU
ESTUDO DE EFICÁCIA DA
VACINA DO BUTANTAN
INSTITUTO BUTANTAN
SÃO PAULO
GOVERNO DO ESTADO


É O ESTUDO QUE INCLUIU MAIS
VOLUNTÁRIOS NO BRASIL
INSTITUTO BUTANTAN
SÃO PAULO
GOVERNO DO ESTADO

Equipe PROFISCOV

Até o momento 12.508 participantes
Cerca de 700 colaboradores em 16 centros de pesquisa em 8 unidades federativas

Grande SP: HC-FMUSP, II Emílio Ribas, IIEP Albert Einstein, Univ. Municipal de São Caetano do Sul

SP Interior: FAMERP, Unicamp, HC FMRP-USP, Hospital de Amor

Sudeste: UFMG, Fiocruz/Niterói
Centro-Oeste: UnB, UFMT, UFMS

Sul: UFPR, Hospital São Lucas - PUCRS, UFPel

- Equipes de CRO e logística
- Equipes administrativas e de apoio Laboratórios de pesquisa
- Parceiros internacionais
- Comitês de acompanhamento
- Comitês de Ética em Pesquisa (CEP/CONEP)
- ANVISA
- Centro de Ensaios Clínicos e Farmacovigilância – IB

Apoio: Fundação Butantan e FAPESP

INSTITUTO BUTANTAN A serviço da vida
SÃO PAULO GOVERNO DO ESTADO

População de estudo

Único estudo feito exclusivamente em profissionais de saúde

13.060 voluntários cuidando de **pacientes com COVID-19**

O maior desafio para uma vacina

- Muito **alto risco de exposição**
- **Maior** dose **infectante**
- **Detecção precoce** de casos
- **Duas doses** com intervalo de duas semanas

PROFISCOV

INSTITUTO BUTANTAN A serviço da vida
SÃO PAULO GOVERNO DO ESTADO

Definição de caso

Um ou mais sintomas por 2 ou mais dias:

- Febre ou calafrios
- Tosse
- Falta de ar ou dificuldade para respirar
- Fadiga
- Dor muscular
- Cefaleia
- Perda de olfato ou paladar
- Dor de garganta
- Congestão nasal ou coriza
- Náusea ou vômito
- Diarreia

RT-PCR por swab respiratório

PROFISCOV

INSTITUTO BUTANTAN A serviço da vida

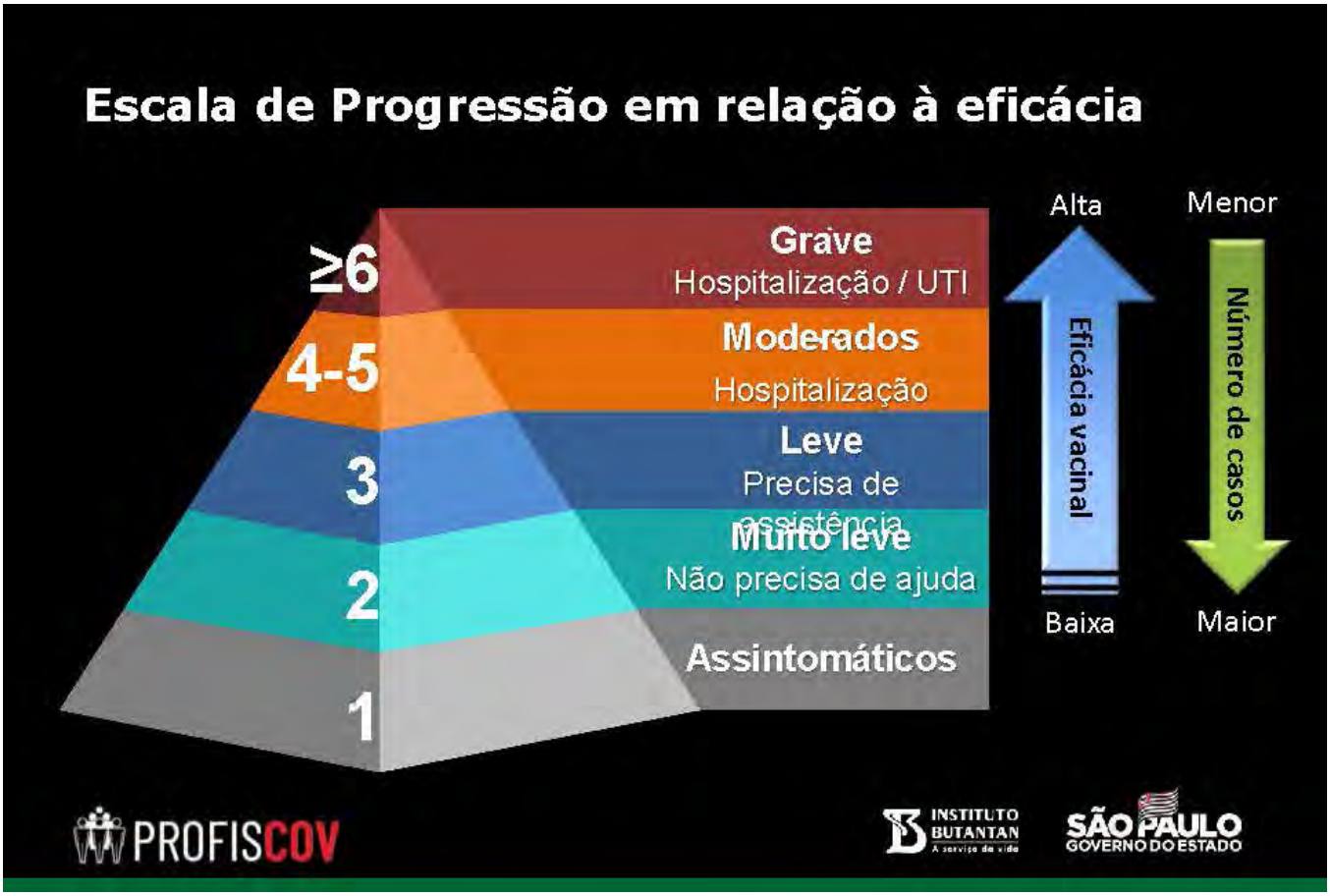
SÃO PAULO GOVERNO DO ESTADO

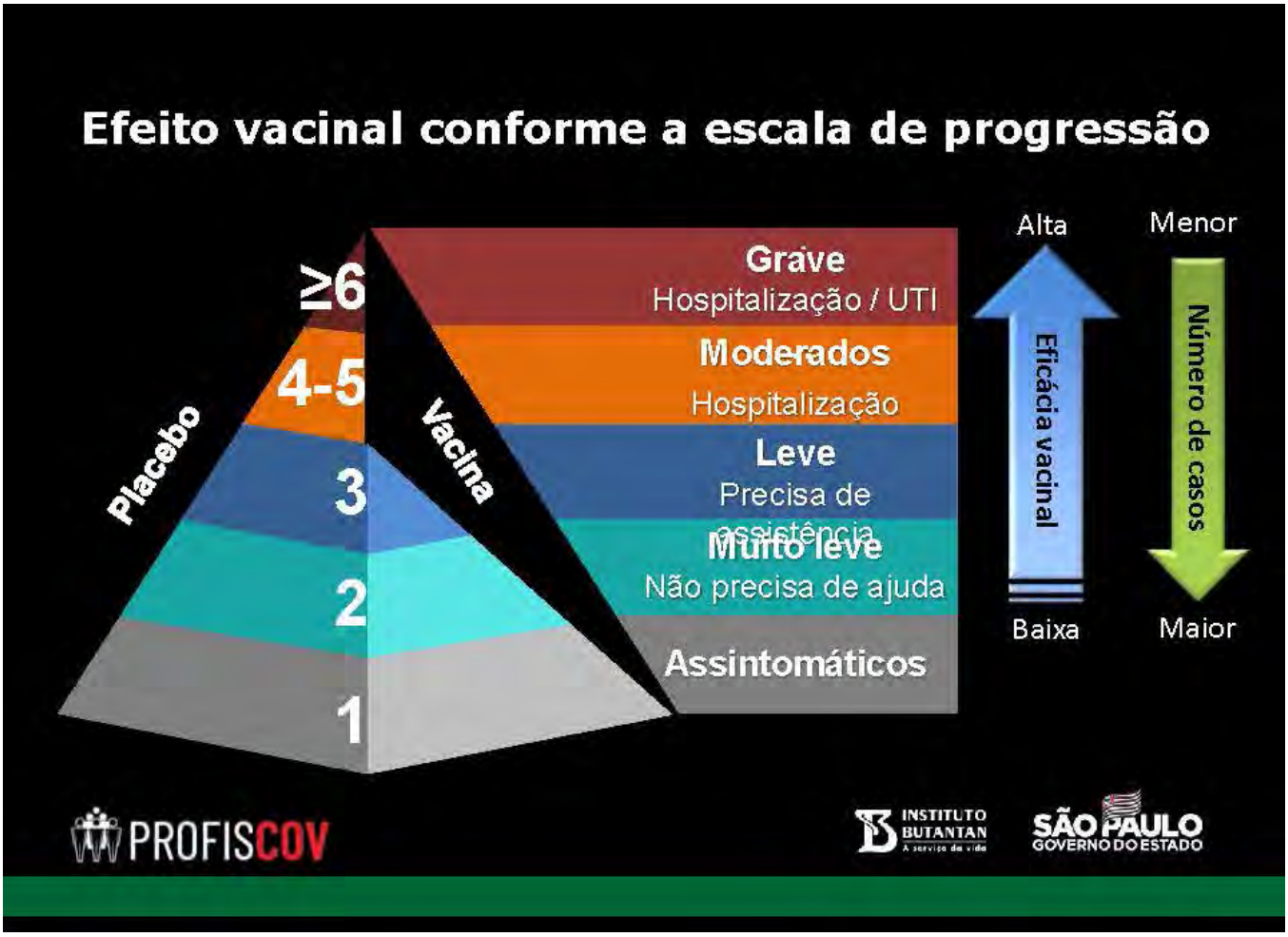
Escala de Progressão da OMS de COVID-19

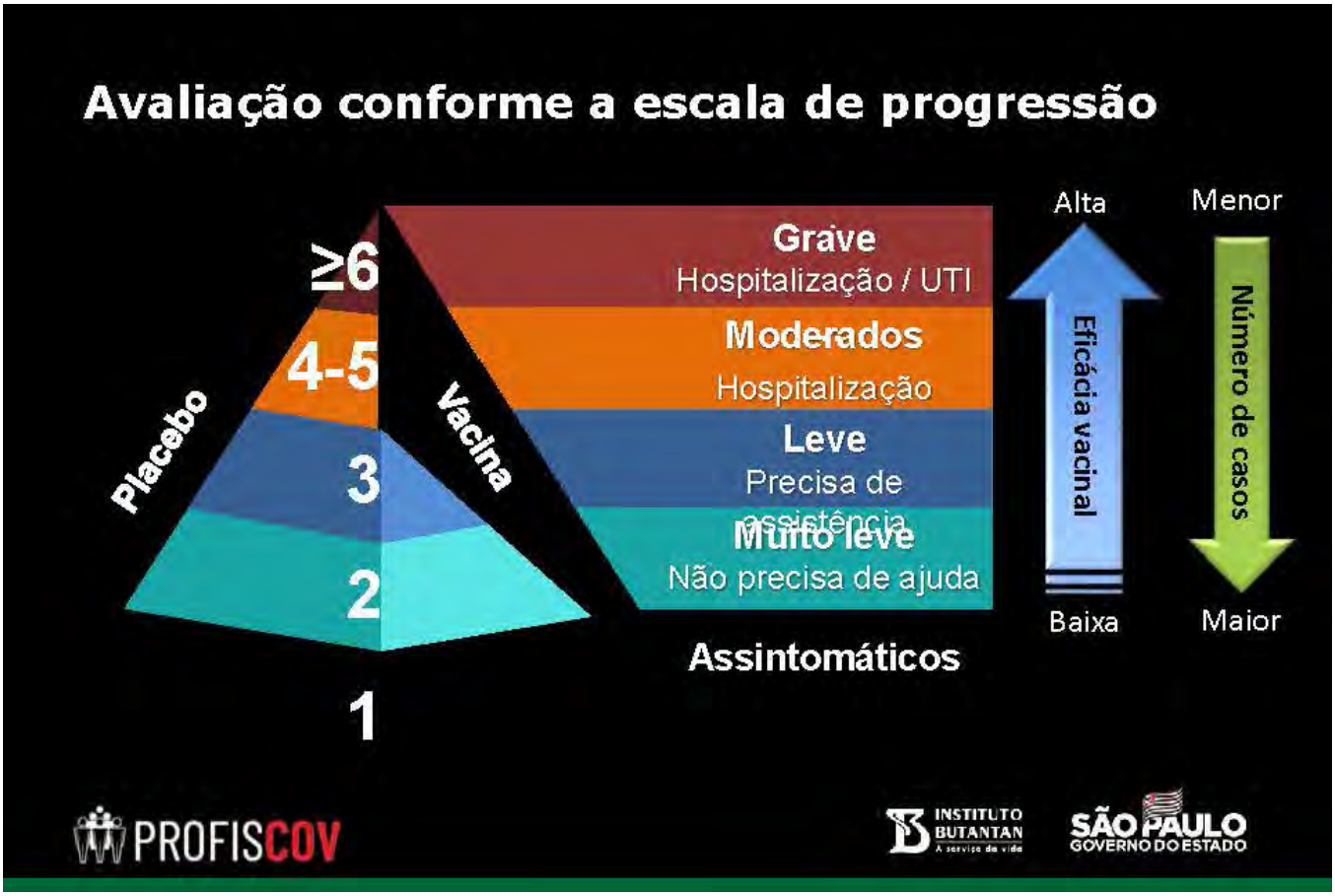
PROFISCOV

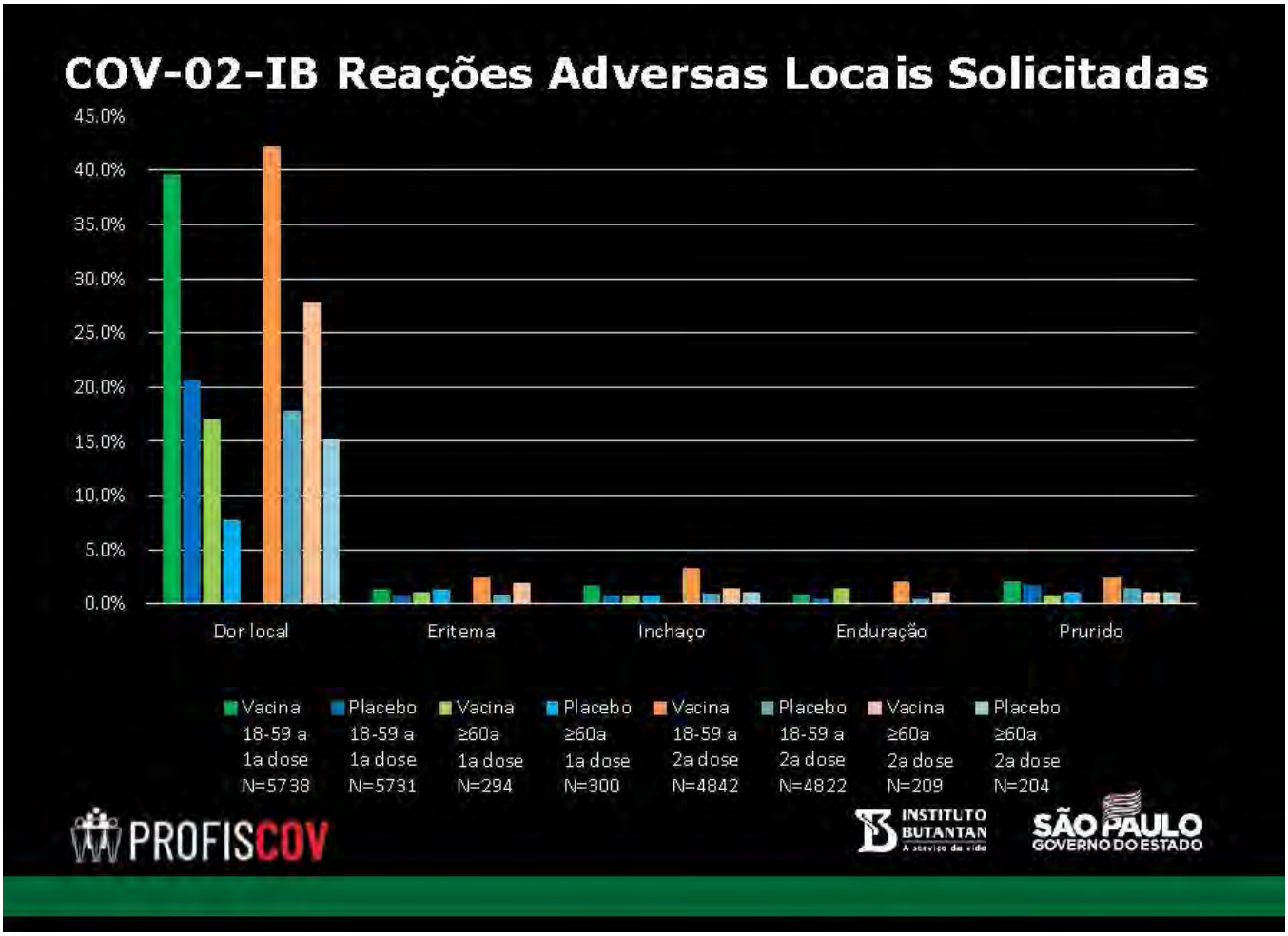
INSTITUTO BUTANTAN A serviço da vida

SÃO PAULO GOVERNO DO ESTADO

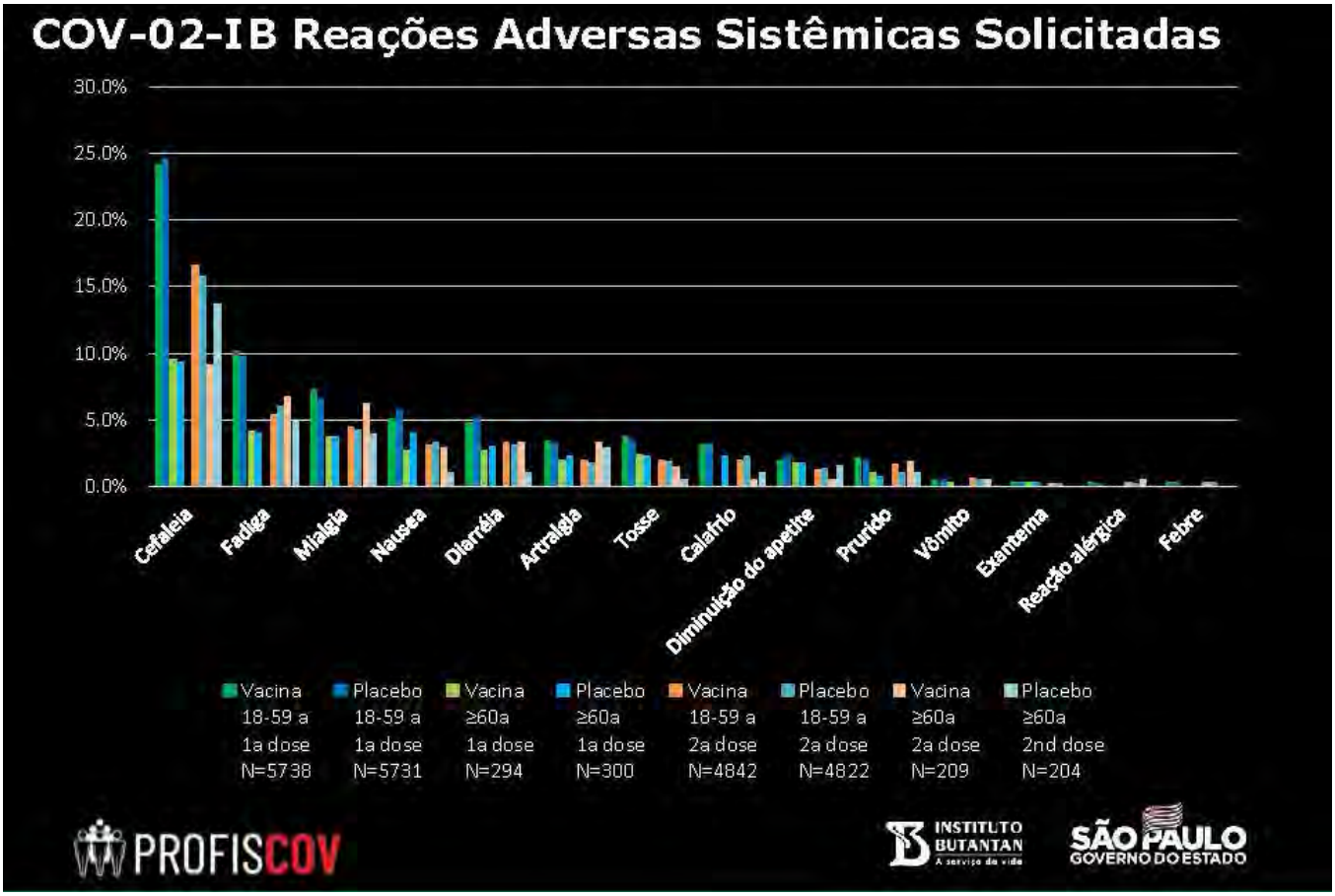

Escala de Progressão em relação à eficácia
≥6
4-5
3
2
1
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
Alta
Baixa
Eficácia vacinal
Menor
Maior
Número de casos
PROFISCOV
INSTITUTO BUTANTAN
SÃO PAULO
GOVERNO DO ESTADO

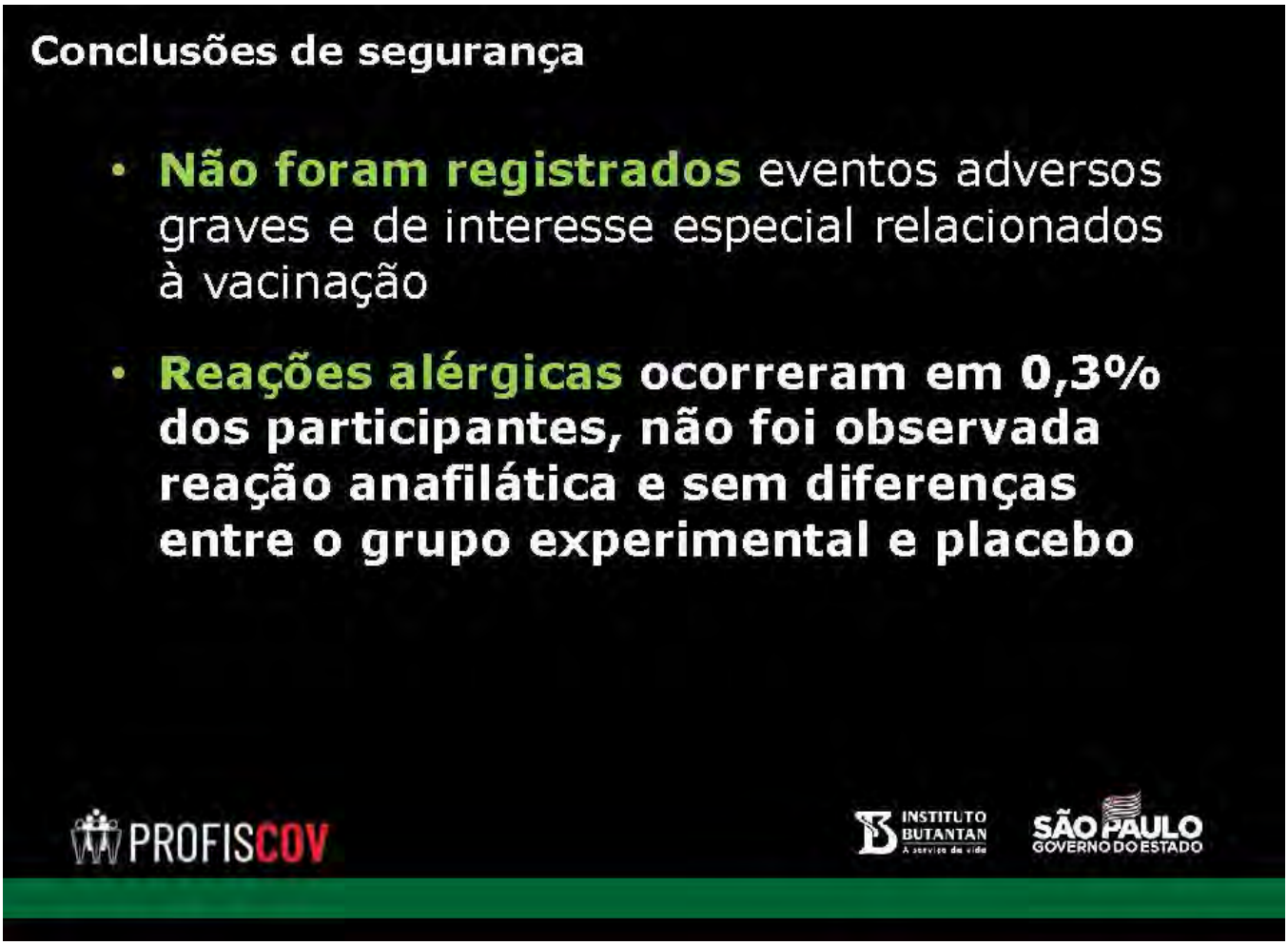

Efeito vacinal conforme a escala de progressão
Placebo
Vacina
≥6
4-5
3
2
1
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
Alta
Baixa
Eficácia vacinal
Menor
Maior
Número de casos
PROFISCOV
INSTITUTO BUTANTAN
SÃO PAULO
GOVERNO DO ESTADO

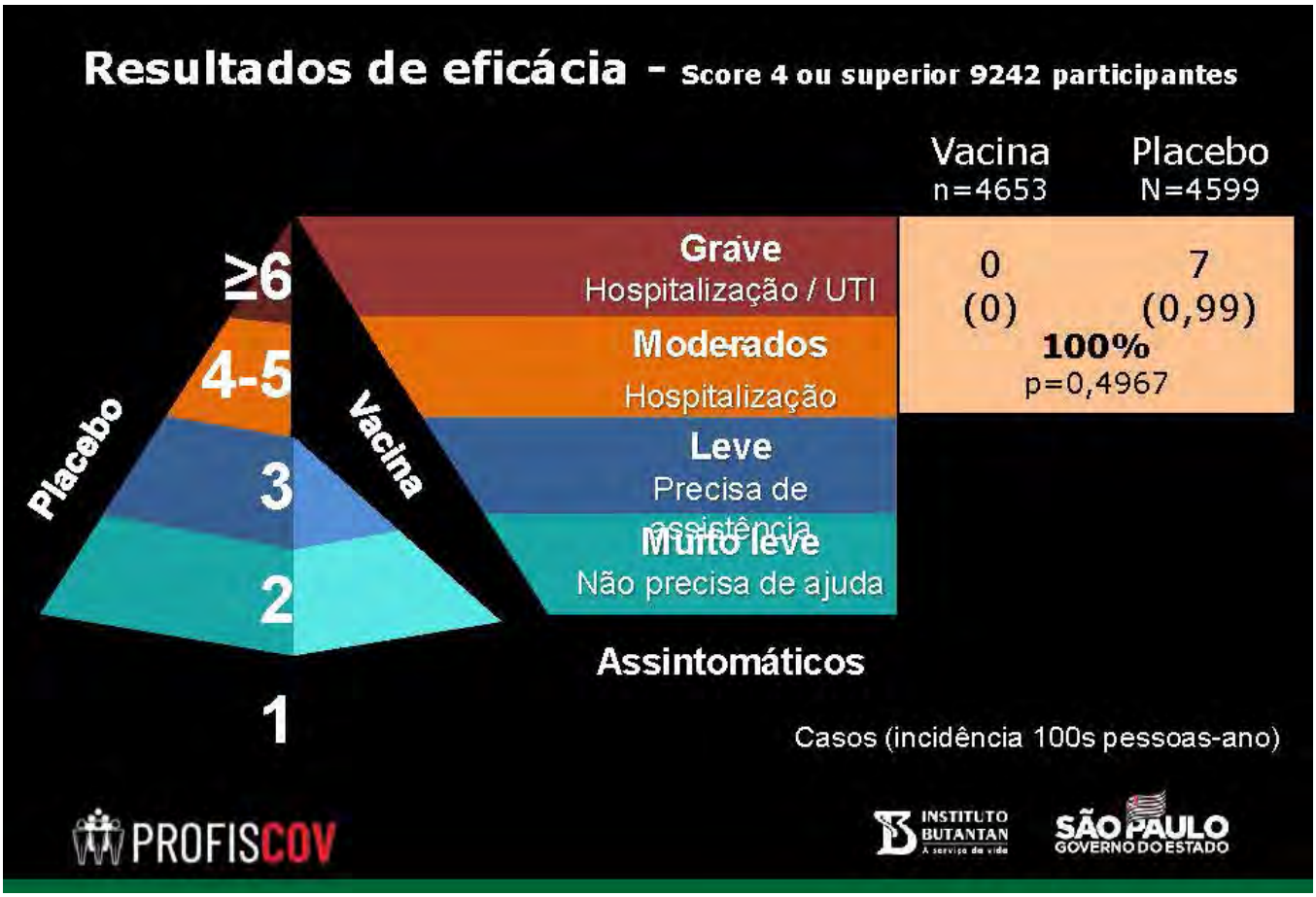

Avaliação conforme a escala de progressão
≥6
4-5
3
2
1
Placebo
Vacina
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
Alta
Baixa
Eficácia vacinal
Menor
Maior
Número de casos
PROFISCOV
INSTITUTO BUTANTAN
SÃO PAULO GOVERNO DO ESTADO

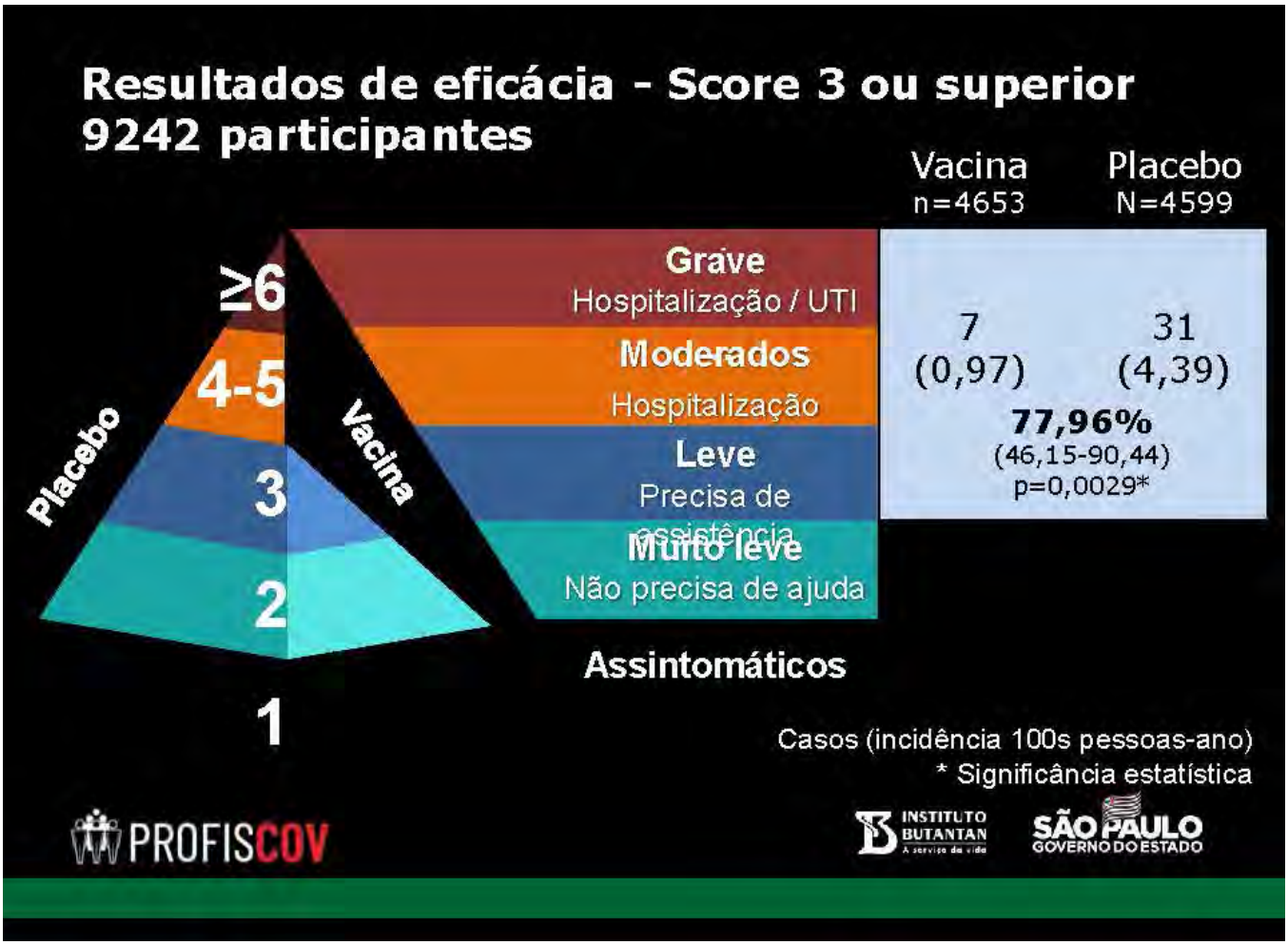

COV-02-IB Reações Adversas Locais Solicitadas
45.0%
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Dor local
Eritema
Inchaço
Enduração
Prurido
Vacina 18-59 a 1a dose N=5738
Placebo 18-59 a 1a dose N=5731
Vacina ≥60a 1a dose N=294
Placebo ≥60a 1a dose N=300
Vacina 18-59 a 2a dose N=4842
Placebo 18-59 a 2a dose N=4822
Vacina ≥60a 2a dose N=209
Placebo ≥60a 2a dose N=204
PROFISCOV
INSTITUTO BUTANTAN
SÃO PAULO GOVERNO DO ESTADO

Estudo

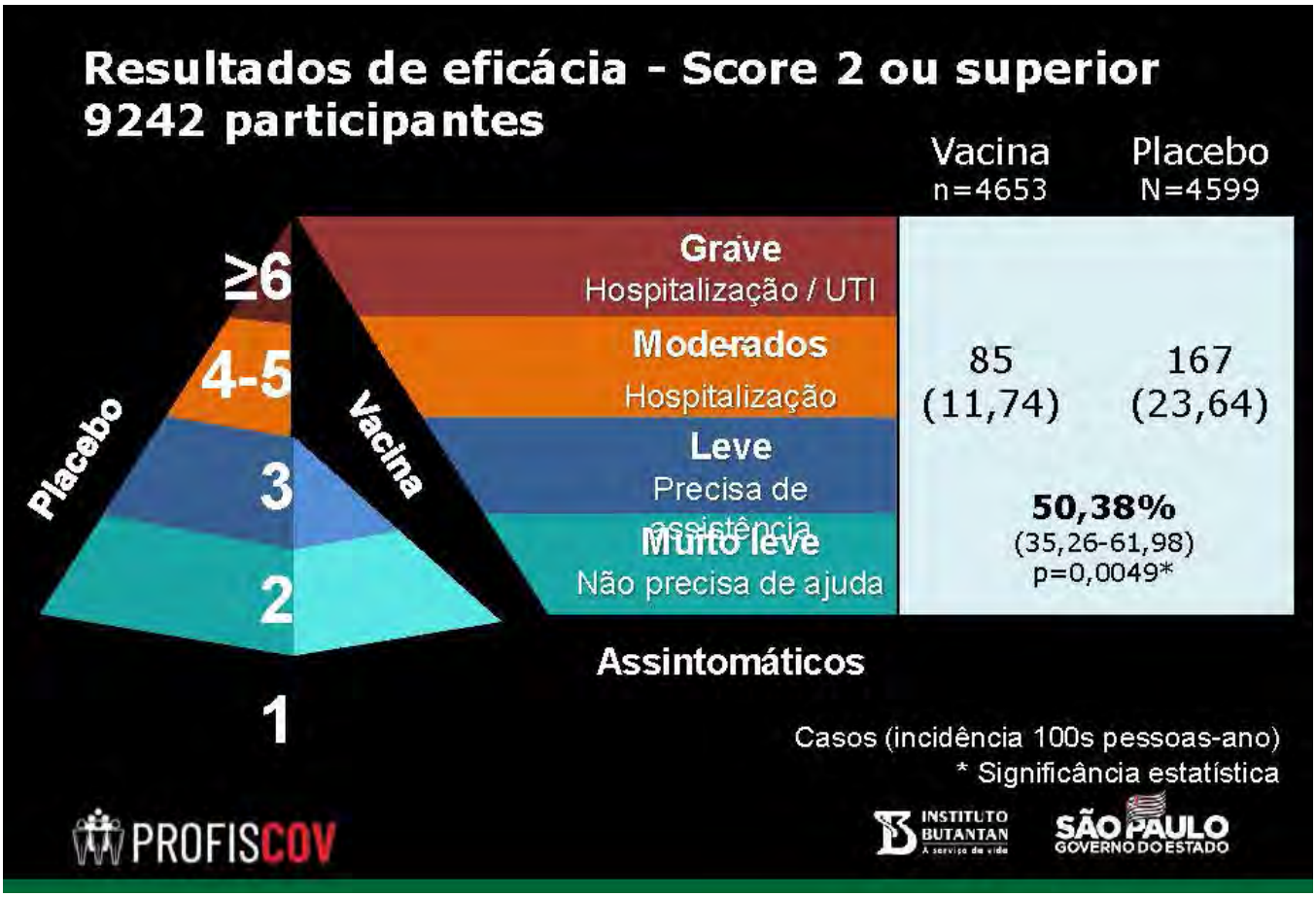

COV-02-IB Reações Adversas Sistêmicas Solicitadas
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Cefaleia
Fadiga
Mialgia
Nausea
Diarréia
Artralgia
Tosse
Calafrio
Diminuição do apetite
Prurido
Vômito
Exantema
Reação alérgica
Febre
Vacina 18-59 a 1a dose N=5738
Placebo 18-59 a 1a dose N=5731
Vacina ≥60a 1a dose N=294
Placebo ≥60a 1a dose N=300
Vacina 18-59 a 2a dose N=4842
Placebo 18-59 a 2a dose N=4822
Vacina ≥60a 2a dose N=209
Placebo ≥60a 2nd dose N=204
PROFISCOV
INSTITUTO BUTANTAN
SÃO PAULO GOVERNO DO ESTADO

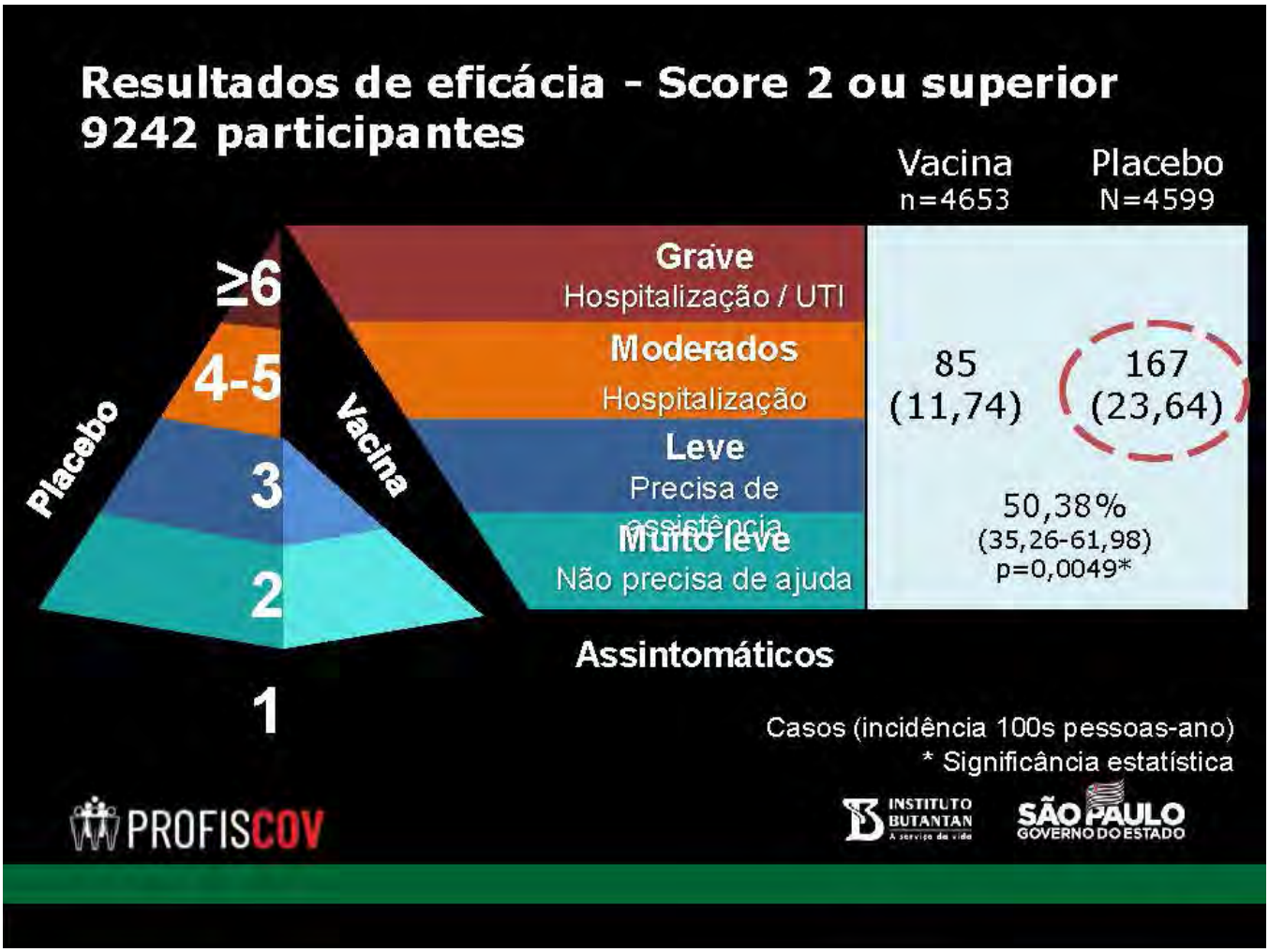

Conclusões de segurança
• Não foram registrados eventos adversos graves e de interesse especial relacionados à vacinação
• Reações alérgicas ocorreram em 0,3% dos participantes, não foi observada reação anafilática e sem diferenças entre o grupo experimental e placebo
PROFISCOV
INSTITUTO BUTANTAN
SÃO PAULO GOVERNO DO ESTADO


Resultados de eficácia - Score 4 ou superior 9242 participantes
Vacina n=4653
Placebo N=4599
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
0 (0)
7 (0,99)
100%
p=0,4967
≥6
4-5
3
2
1
Placebo
Vacina
Casos (incidência 100s pessoas-ano)
PROFISCOV
INSTITUTO BUTANTAN
SÃO PAULO GOVERNO DO ESTADO


Resultados de eficácia - Score 3 ou superior 9242 participantes
Vacina n=4653
Placebo N=4599
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
7 (0,97)
31 (4,39)
77,96%
(46,15-90,44)
p=0,0029*
≥6
4-5
3
2
1
Placebo
Vacina
Casos (incidência 100s pessoas-ano)
* Significância estatística
PROFISCOV
INSTITUTO BUTANTAN
SÃO PAULO GOVERNO DO ESTADO

Estudo


Resultados de eficácia - Score 2 ou superior
9242 participantes
Vacina
n=4653
Placebo
N=4599
≥6
4-5
3
2
1
Placebo
Vacina
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
85
(11,74)
167
(23,64)
50,38%
(35,26-61,98)
p=0,0049*
Casos (incidência 100s pessoas-ano)
* Significância estatística
PROFISCOV
INSTITUTO BUTANTAN
SÃO PAULO
GOVERNO DO ESTADO


Resultados de eficácia - Score 2 ou superior
9242 participantes
Vacina
n=4653
Placebo
N=4599
≥6
4-5
3
2
1
Placebo
Vacina
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
85
(11,74)
167
(23,64)
50,38%
(35,26-61,98)
p=0,0049*
Casos (incidência 100s pessoas-ano)
* Significância estatística
PROFISCOV
INSTITUTO BUTANTAN
SÃO PAULO
GOVERNO DO ESTADO

Resumo de resultados de eficácia
9242 participantes

* Significância estatística

PROFISCOV

INSTITUTO BUTANTAN
A serviço da vida

SÃO PAULO
GOVERNO DO ESTADO

Conclusões e perspectivas

- A vacina COVID-19 do Butantan é **muito segura**
- A eficácia vacinal para diminuir a doença clínica foi demonstrada em **situação de alta exposição**
- O efeito tende a aumentar conforme aumenta a **intensidade da doença**
- O uso da vacina pode **evitar** que os **casos de COVID-19** precisem de **assistência ambulatorial ou hospitalar**
- O efeito em **uso comunitário** pode ser **ainda maior**
- O efeito sobre **transmissão** precisara de avaliação em **novo estudo**

INSTITUTO BUTANTAN
A serviço da vida

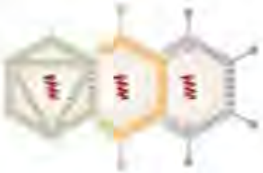
SÃO PAULO
GOVERNO DO ESTADO

Estratégias

Vírus atenuado, morto ou VLP

Proteínas virais

Vetores virais



RNAm e DNA

https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html

INSTITUTO BUTANTAN A serviço da vida

SÃO PAULO GOVERNO DO ESTADO

Desenvolvimento

	U. Oxford / Astra Zeneca
	CanSino / Beijing Inst Tech
	Gamaleya
	Jansen / Johnson & Johnson
Novavax	Sinovac
AnGes / Osaka University / Takara Bio	Wuhan Inst Biol / Sinopharm
Bektop	Beijing Inst Biol / Sinopharm
Anhui Zhifei Longcom	Barath
Medicago / GSK	Moderna / NIAID
Clover / GSK / Dynavax	BioNTech / Pfizer
Murdoch Children's Research Institute	Curevac

172	43	20	20	8	2	1
Pré-Clínico	Fase 1	Fase 2	Fase 3	Acesso limitado	Aprovada	Suspensa

https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines
https://www.nytimes.com/interactive/2020/science/coronavirus vaccine tracker.html

INSTITUTO BUTANTAN A serviço da vida

SÃO PAULO GOVERNO DO ESTADO

Eficácia dos estudos

- Pfizer **95%**
- Moderna **94,1%**
- Gamaleya **90%**
- Sinopharm **79%**
- AstraZeneca **62 a 90%**

INSTITUTO BUTANTAN
A serviço da vida

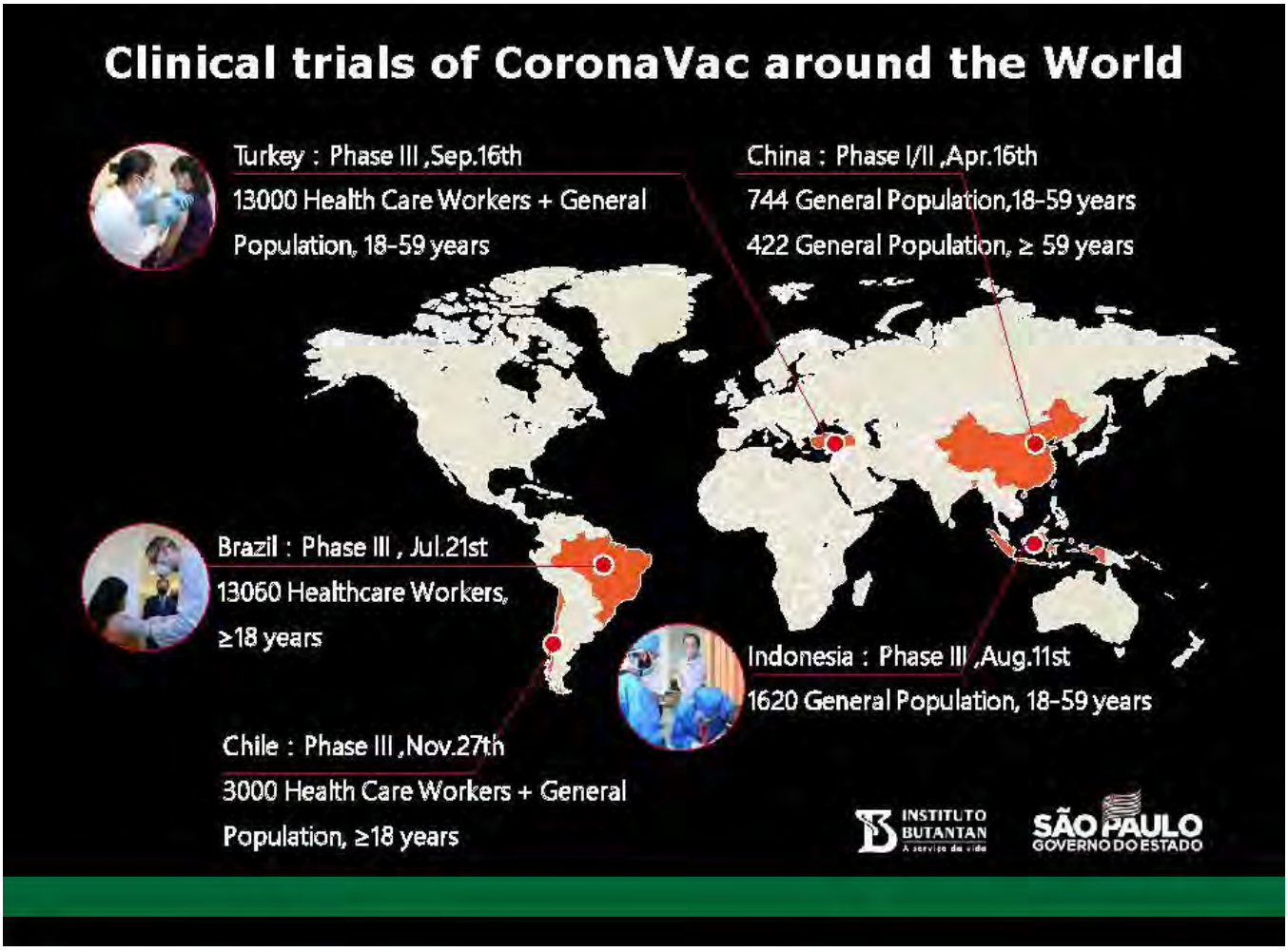
SÃO PAULO
GOVERNO DO ESTADO

Eficácia dos estudos

- Pfizer 95%
- Moderna 94,1%
- AstraZeneca 62 a 90%
- Sinopharm 79%
- Gamaleya 90%
- Sinovac 50,3 a 100%

Pfizer			
Total	90.3	95	97.6
OMS 4		100	
Moderna			
Total	89.3	94.1	96.8
OMS 4		100	
AstraZeneca			
Total	54.8	70.4	80.6
BD/AD	67.4	90	97
AD/AD	28	60.3	78.2
OMS 4			
Sinopharm			
Total		79	
Gamaleya			
Total		91.4	
Sinovac			
OMS 2	35.26	50.39	61.98
OMS 3	49.15	77.96	90.44
OMS 4	95.42	100	100

Nenhuma das vacinas foi testada em um ambiente de incidência tão alta



Result of Phase I/II Study in China

1. Safety

No serious adverse reactions were observed in both vaccine group and placebo group

There was no significant difference between vaccine and placebo groups regarding to adverse reactions after vaccination

Majority of adverse reactions were grade 1

2.Immunogenicity

CoronaVac induced no less than 94.9% seroconversion of neutralizing antibody.

Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2020 Nov 17

INSTITUTO BUTANTAN A serviço da vida

SÃO PAULO GOVERNO DO ESTADO

Result of Phase III Study in Turkey

1. Safety

There was no significant difference between vaccine and placebo groups regarding to adverse events after vaccination

Majority of adverse events were grade 1

No Vaccine-enhanced disease (VED) was observed

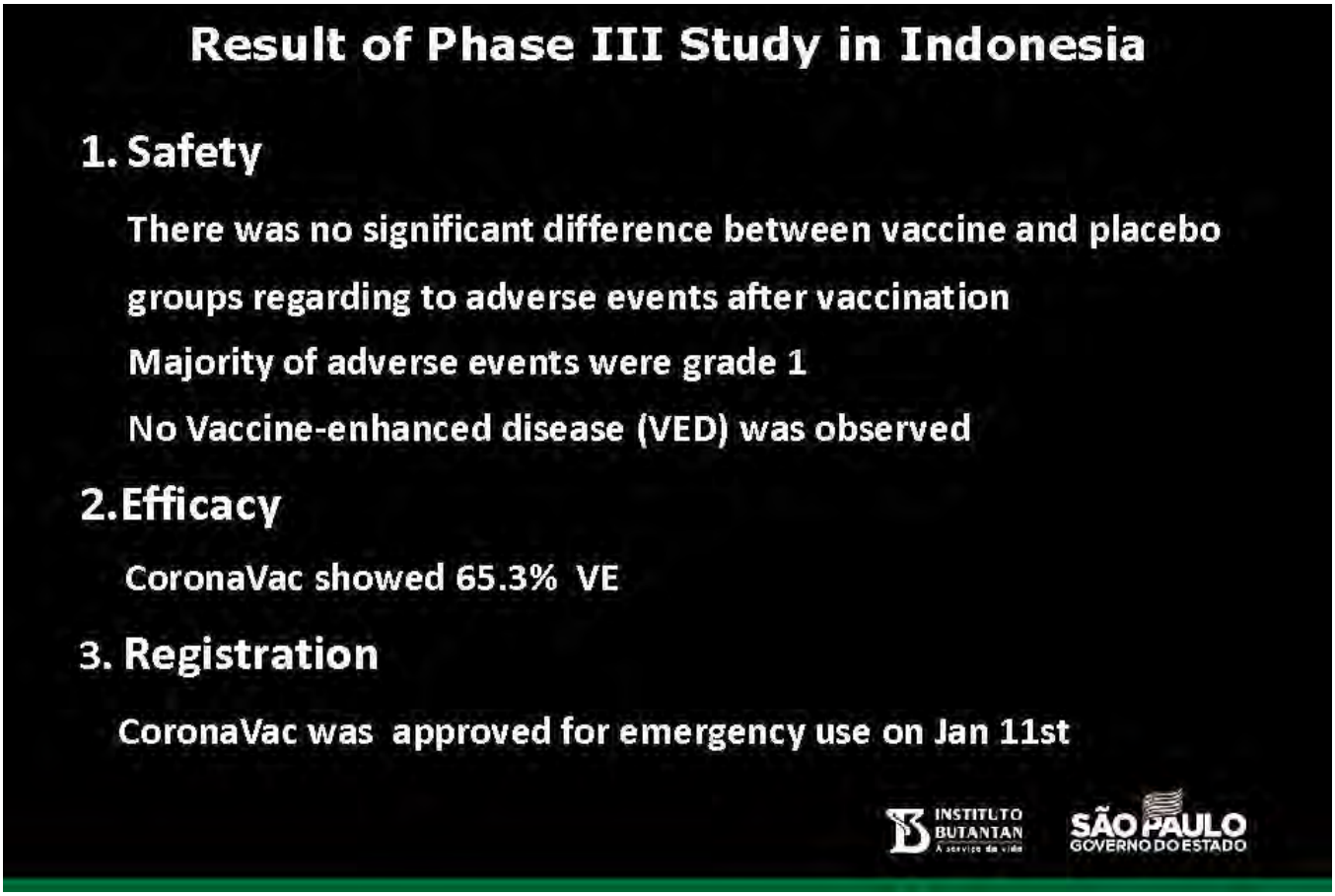
2. Efficacy

CoronaVac showed 91.25% VE

100% protection for hospitalized cases

INSTITUTO BUTANTAN A serviço da vida

SÃO PAULO GOVERNO DO ESTADO


Result of Phase III Study in Indonesia
1. Safety
There was no significant difference between vaccine and placebo groups regarding to adverse events after vaccination
Majority of adverse events were grade 1
No Vaccine-enhanced disease (VED) was observed
2. Efficacy
CoronaVac showed 65.3% VE
3. Registration
CoronaVac was approved for emergency use on Jan 11st
INSTITUTO BUTANTAN
SÃO PAULO GOVERNO DO ESTADO


SÃO PAULO
GOVERNO DO ESTADO
Obrigado

1.19. Estudos confirmam segurança da vacina contra coronavírus desenvolvida em parceria com o Butantan

A vacina CoronaVac, desenvolvida em parceria com o Instituto Butantan, se mostrou segura e com bom índice de imunogenicidade. A constatação é de um estudo publicado pela farmacêutica chinesa Sinovac Life Science. A pesquisa analisou o comportamento de 600 voluntários vacinados na China durante a fase 2 dos testes clínicos.

Cada voluntário recebeu duas doses, sendo que metade dos participantes tomaram a vacina propriamente dita e a outra metade placebo. De acordo com o que foi identificado nos estudos, não existe nenhuma preocupação com relação a segurança da vacina utilizada nos voluntários. Dentre as principais reações está leve dor no local da aplicação.

A vacina desenvolvida pela Sinovac Life Science é uma das mais promissoras do mundo porque utiliza tecnologia já conhecida e amplamente aplicada em outras vacinas. O Instituto Butantan avalia que sua incorporação ao sistema de saúde deva ocorrer mais facilmente.

O laboratório asiático já realizou testes em cerca de mil voluntários na China, nas fases 1 e 2. Antes, o modelo experimental aplicado em macacos apresentou resultados expressivos em termos de resposta imune contra o coronavírus.

A farmacêutica forneceu ao Butantan as doses da vacina para a realização de testes clínicos de fase 3 em voluntários no Brasil, com o objetivo de demonstrar sua eficácia e segurança.

Caso a vacina seja aprovada, será realizada a transferência de tecnologia para produção em escala e fornecimento gratuito pelo SUS. Os passos seguintes serão o registro do imunizante pela Anvisa (Agência Nacional de Vigilância Sanitária) e distribuição em todo o Brasil.

Publicado em: 11/08/2020

Full title

Immunogenicity and Safety of a SARS-CoV-2 Inactivated Vaccine in Healthy Adults Aged 18-59 years: Report of the Randomized, Double-blind, and Placebo-controlled Phase 2 Clinical Trial

Running title

Phase 2 Clinical Trial of SARS-CoV-2 Inactivated Vaccine

Author List:

Yanjun Zhang*, Ph.D., zhangyjtt@126.com, Department of Microbiology, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China.

Gang Zeng*, Ph.D., zengg@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Hongxing Pan*, M.Sc., panhongxing@126.com, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.

Changgui Li*, Ph.D., changguili@aliyun.com, National Institutes for Food and Drug Control, Beijing.

Biao Kan*, Ph.D., kanbiao@icdc.cn, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing, China.

Yaling Hu*, M.Sc., huyl@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Haiyan Mao, M.Sc., hymao@cdc.zj.cn, Department of Microbiology, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China.

Qianqian Xin, Ph.D., xinqq@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Kai Chu, M.Sc., chukai19812007@163.com, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.

Weixiao Han, M.Sc., hanwx@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Zhen Chen, M.Sc., robbieagassi@hotmail.com, National Institutes for Food and Drug Control, Beijing.

Rong Tang, B.A., tangrongtr@126.com, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.

Weidong Yin, MBA, yinwd@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Xin Chen, B.A., kjhszxchenxin@163.com, Suining County Center for Disease Control and Prevention, Suining, Jiangsu Province, China.

Xuejie Gong, B.A., gongxj@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Chuan Qin, Ph.D., qinchuan@pumc.edu.cn, Key Laboratory of Human Disease Comparative Medicine, Chinese Ministry of Health, Beijing Key Laboratory for Animal Models of Emerging and Remerging Infectious Diseases, Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Comparative Medicine Center, Peking Union Medical College, Beijing, China.

Yuansheng Hu, M.P.H., huys@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Xiaoyong Liu, B.A., 317840532@qq.com, Suining County Center for Disease Control and Prevention, Suining, Jiangsu Province, China.

Guoliang Cui, B.A., cuigl@sinovac.com, Sinovac Life Sciences Co., Ltd., Beijing,

China.

Congbing Jiang, B.A., snjkws@126.com, Suining County Center for Disease Control and Prevention, Suining, Jiangsu Province, China.

Hengming Zhang, M.Sc., zhanghm@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Jingxin Li, Ph.D., jingxin42102209@126.com, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.

Minnan Yang, Ph.D., 28453073@qq.com, CAS Key Laboratory of Infection and Immunity, National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Xiaojuan Lian, MBA, lianxj@sinovac.com, Sinovac Life Sciences Co., Ltd., Beijing, China.

Yan Song, B.A., songyanww@126.com, Suining County Center for Disease Control and Prevention, Suining, Jiangsu Province, China.

Jinxing Lu¶, Ph.D., lujinxing@icdc.cn, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing, China.

Xiangxi Wang¶, Ph.D., xiangxi@ibp.ac.cn, CAS Key Laboratory of Infection and Immunity, National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Miao Xu¶, Ph.D., xumiaobj@126.com, National Institutes for Food and Drug

Control, Beijing.

Qiang Gao¶, M.Sc., gaoq@sinovac.com, Sinovac Life Sciences Co., Ltd., Beijing, China.

Fengcai Zhu¶, M.D., jszfc@vip.sina.com, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.

Footnote

*The first six authors, YZ, GZ, HP, CL, BK, and YH, contribute equally to the manuscript and are listed as the first authors.

¶The last five authors, JL, XW, MX, QG, and FZ, contribute equally to the correspondence and are listed as the corresponding authors.

Clinical Trial number: NCT04352608

Funding Project:

National Key Research and Development Program (2020YFC0849600)

Beijing Science and Technology Program (Z201100005420023)

78 **ABSTRACT**

79 BACKGROUND

80 The top priority for the control of COVID-19 pandemic currently is the development
81 of a vaccine. A phase 2 trial conducted to further evaluate the immunogenicity and
82 safety of a SARS-CoV-2 inactivated vaccine (CoronaVac).

83 METHODS

84 We conducted a randomized, double-blind, placebo-controlled trial to evaluate the
85 optimal dose, immunogenicity and safety of the CoronaVac. A total of 600 healthy
86 adults aged 18-59 years were randomly assigned to receive 2 injections of the trial
87 vaccine at a dose of 3 μg/0.5 mL or 6 μg /0.5mL, or placebo on Day 0,14 schedule or
88 Day 0,28 schedule. For safety evaluation, solicited and unsolicited adverse events
89 were collected after each vaccination within 7 days and 28 days, respectively. Blood
90 samples were taken for antibody assay.

91 RESULTS

92 CoronaVac was well tolerated, and no dose-related safety concerns were observed.
93 Most of the adverse reactions fell in the solicited category and were mild in severity.
94 Pain at injection site was the most frequently reported symptoms. No Grade 3 adverse
95 reaction or vaccine related SAEs were reported. CoronaVac showed good
96 immunogenicity with the lower 3 μg dose eliciting 92.4% seroconversion under Day
97 0,14 schedule and 97.4% under Day 0,28 schedule. 28 days after two-dose
98 vaccination, the Nab levels of individual schedules range from 23.8 to 65.4 among
99 different dosage and vaccination schedules.

100 CONCLUSIONS

101 Favorable safety and immunogenicity of CoronaVac was demonstrated on both
102 schedules and both dosages, which support the conduction of phase 3 trial with
103 optimum schedule/dosage per different scenarios.

104 **Keywords:** COVID-19; SARS-CoV-2; Inactivated vaccine; Clinical Trial.

BACKGROUND

In January 2020, outbreaks of coronavirus disease in 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) escalated rapidly, and since then COVID-19 cases have been reported in over 200 countries and territories. The pandemic continues to spread unabated affecting the health and changing the lifestyles of people globally.[1] To reduce the disease burden and stop the community-wide transmission of COVID-19 across the globe, specific therapeutic agents or vaccines are urgently needed. Till now, more than 120 vaccine candidates have been reported to be under development and at least 23 have progressed to the clinical evaluation stage.[2]

The inactivated SARS-CoV-2 vaccine with aluminum hydroxide developed by Sinovac Life Sciences Co., Ltd., also known as CoronaVac, has been shown to be safe and could induce SARS-CoV-2 specific neutralizing antibodies in mice, rats, and nonhuman primates.[3] On the basis of the results obtained from our phase 1 trial, no safety concerns have been identified. Notably, immunization of CoronaVac induced immune responses against SARS-CoV-2 in adults. Here, we report the results of the phase 2 trial.

METHODS

TRIAL DESIGN AND OVERSIGHT

This double-blind, randomized and placebo-controlled phase 2 clinical trial based on a seamless design was registered at clinicaltrials.gov (NCT04352608) and was conducted in Suining County, Jiangsu Province, China. Detailed information about the trial has been provided in our previous phase 1 study. The trial protocol and the

informed-consent form were approved by the ethics committee of the Jiangsu Provincial Center for Disease Control and Prevention (JSCDC). This clinical trial was conducted in accordance with the Chinese regulatory requirements and the standards of good clinical practice.

Before enrollment, written informed consent was obtained from each participant. The main exclusion criteria included high-risk epidemiological history, positive IgG, IgM or nucleic acid test of pharyngeal or anal swab, axillary temperature >37.0□, allergy to a vaccine component, and other unsuitable conditions.

A total of 600 healthy adults aged 18-59 years were randomly assigned into 3 groups in a ratio of 2:2:1 to receive 2 injections of the trial vaccine at a dose of 3 μg/0.5 mL or 6 μg /0.5mL, or placebo on a Day 0,14 schedule or a Day 0,28 schedule, according to a random list generated by an independent statistician..

VACCINE

The vaccine candidate was an inactivated SARS-CoV-2 whole virion vaccine with aluminium hydroxide as adjuvant (CoronaVac) developed by Sinovac Life Sciences Co., Ltd. SARS-CoV-2 virus was propagated in Vero cells and harvested. The harvested virus was inactivated using β-propiolactone and further purified. The bulk vaccine material obtained from this step was then adsorbed onto aluminium hydroxide and formulated with phosphate-buffered saline (PBS) and sodium chloride as inactivated final product. The dosage of 3 μg/0.5 mL and 6 μg /0.5mL were adopted in this study. Whereas the placebo contained aluminum hydroxide diluents with no antigen. Both were administered intramuscularly on the schedule of Day 0,14 or Day 0,28.

SAFETY ASSESSMENT

For safety evaluation of CoronaVac, the participants who received at least one dose of vaccination was included. All vaccinated subjects were observed for immediate adverse events (AEs) on-site for at least 30 minutes after each administration. Diary cards were issued to the participants to record the solicited AEs (e.g. pain, induration, swelling, redness, rash, pruritus) occurring on day 0~7 and unsolicited AEs (e.g. fever, acute allergic reaction, skin and mucosa abnormality, diarrhea, anorexia, vomiting, nausea, muscle pain, headache, cough, fatigue) occurring on day 0~28. Data on serious adverse events (SAEs) were collected throughout the trial. All AEs were assessed for severity, and the relationship to vaccination was decided by investigators before unblinding.

IMMUNOGENICITY

To assess immune response, blood samples were collected from each participant different time points (0/28/42th day for Day 0,14 schedule, and 0/56th day for Day 0,28 schedule). The ability of the antibodies present in the blood sample to bind the receptor binding domain (RBD) of SARS-CoV-2 was assessed by enzyme-linked immunosorbent assay (ELISA). A dilution of 1:160 was considered as a positive cutoff value. We also measured neutralizing antibody titer (Nab) using a modified cytopathogenic effect assay. A titer of 1:8 or higher indicated seropositivity. Seroconversion was defined as a change from seronegative (<1:8) to seropositive (≥ 1:8) or a 4-fold increase from baseline titers if seropositive.

The neutralizing antibody assay was performed by Chinese National Institutes for Food and Drug Control, and the ELISA was performed by Sinovac Biotech.

NEGATIVE STAIN

Virus particles of vaccine used for phase 1 and 2 were diluted to a concentration of 0.04 mg/mL, deposited on a glow-discharged carbon-coated copper grid (Electron Microscopy Sciences) and after 1 min, washed twice with buffer (20 mM Tris, 200 mM NaCl, pH 8.0), and stained with 1% phosphotungstic acid (pH 7.0) for 1 min. Then the grid was imaged at room temperature using FEI Tecnai Spirit electron microscope (Thermo Fisher Scientific) operated at an acceleration voltage of 120 kV.

STATISITICAL ANALYSIS

Safety evaluation was performed on participants who received at least 1 dose of the vaccine or placebo by comparing the overall incidence rate of solicited and unsolicited AEs among relevant groups. Immunogenicity assessment was performed on the per-protocol set (PPS). The seroconversion rate was defined as a change from seronegative to seropositive or a 4-fold increase from baseline titers if seropositive. The titer distributions were described with reverse cumulative distribution curves and were tested with the nonparametric Kruskal-Wallis test over the groups.

The Pearson Chi-square test or Fisher's exact test was adopted for the analysis of binary outcomes. Clopper-Pearson method was used to compute the 95% confidence intervals (CIs) of the binary outcome. ANOVA method was utilized to compare the GMTs among groups. Hypothesis testing was two-sided with an alpha value of 0.05. Analyses were conducted by SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

196 **STUDY POPULATION**

197 From 29 April to 5 May 2020, 600 subjects were enrolled and randomly assigned to
198 receive first of the CoronaVac or placebo dose. All subjects were included into the
199 safety assessment. During this trial, 297 subjects put on Day 0,14 schedule and 294
200 subjects following Day 0,28 schedule were included in the per-protocol cohort for
201 immunogenicity analysis. These subjects received the 2 injections, attended all visits
202 and gave planned blood sample. Information about study enrollment, randomization,
203 and vaccination is shown in Fig. S1.

204 Baseline demographic characteristics at enrollment were similar among these groups
205 in terms of sex, mean age, height, and weight (Table 1).

206

207

208 **Table 1. Baseline Characteristics of the Study Participants.***

Characteristics	3 μg Group	6 μg Group	Placebo	*P*
Day 0,14 schedule				
N	120	120	60	
Age (years)	42.0±10.2	42.4±9.0	43.6±7.6	0.5543
Gender (male/female)	54/66	48/72	25/35	0.7305
Height (m)	1.7±0.1	1.6±0.1	1.6±0.1	0.3864
Body weight (kg)	67.8±11.7	68.7±11.5	68.4±10.9	0.8258
BMI (kg/m2)	24.9±3.6	25.5±3.2	25.5±3.0	0.2930
Day 0,28 schedule				
N	120	120	60	

Age (years)	41.5±9.6	40.6±9.9	44.3±8.4	0.0472
Gender (male/female)	63/57	63/57	30/30	0.9417
Height (m)	1.7±0.1	1.7±0.1	1.7±0.1	0.9433
Body weight (kg)	70.0±11.8	70.0±12.2	72.1±12.2	0.4704
BMI (kg/m2) §	25.2±3.1	25.2±3.3	26.1±3.1	0.1741

209 * Plus-minus values are means ±SD.

210 § BMI=body mass index.

211

212 **ADVERSE REACTIONS**

213 For subjects in Day 0,14 schedule, the incidence rates of adverse reactions in 6 μg, 3
214 μg and placebo group were 35.0%, 33.3% and 21.7%, respectively; while the
215 corresponding incidence rates were 19.2%, 19.2% and 18.3% in Day 0,28 schedule,
216 respectively. Within each schedule, there was no significant difference in the
217 occurrence of adverse reactions among all vaccine and placebo groups (Fig. 1). Most
218 of the adverse reactions were solicited adverse reactions and mild in severity. After
219 each injection, pain at the injection site was the most frequently reported local
220 symptoms, which reported in 61 subjects (20.3%) on Day 0,14 schedule and 31
221 subjects (10.3%) on Day 0, 28 schedule. (Additional detailed results related to adverse
222 reactions are available in Table S1).

223 We did not observe any Grade 3 adverse reaction. Most reported adverse reactions
224 resolved within 72 hours after vaccine administration. During the follow-up period, 3
225 SAEs were reported from 3 subjects and neither was vaccine related.

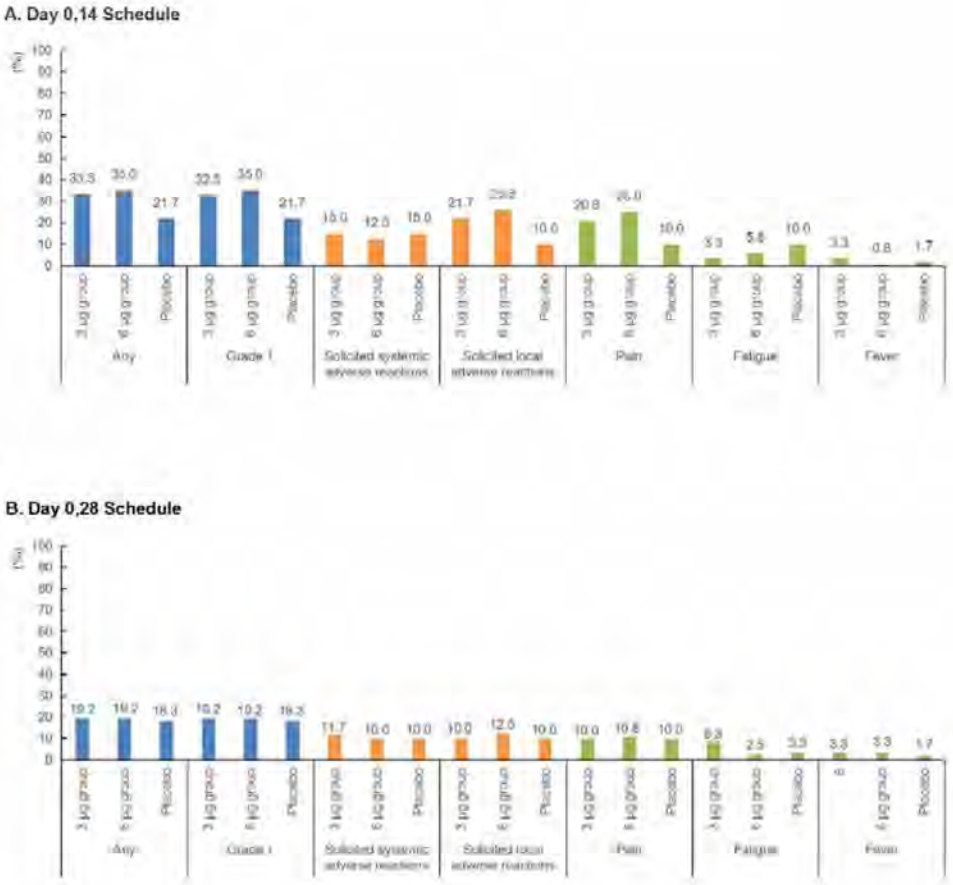


Figure legends

Figure 1. Incidence rates of adverse reactions among different groups in phase 2.

(A) The incidence rates of adverse reactions among different groups with a Day 0,14 schedule. (B) The incidence rates of adverse reactions among different groups with a Day 0,28 schedule.

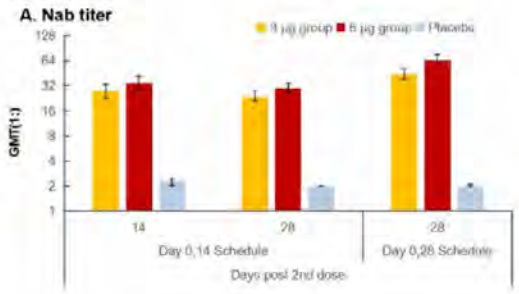
IMMUNOGENICITY

At baseline, all the 600 subjects were seronegative (with Nab titers of <1:8); but the seroconversion rates increased over 90% during the later stages of the trial. Within

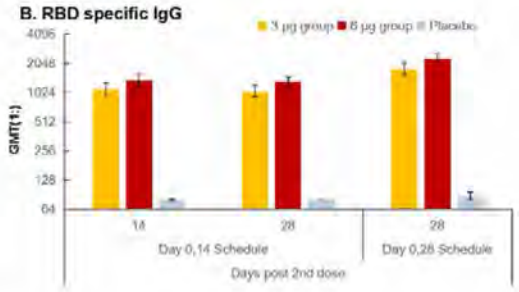
each dosage, there was no significant difference in the seroconversion rates between Day 0,14 and Day 0,28 schedule. For the antibody response against the receptor binding domain, similar results were observed (Table S2). No changes in seropositivity frequencies and GMTs from baseline were found for the placebo group.

For subjects on Day 0,14 schedule, the GMT increased to 34.5 (95% CI, 28.5 to 41.8) and 27.6 (95% CI, 22.7 to 33.5) in 6 μg and 3 μg group, respectively, and remained stable after 28 days from the second injection (Fig. 2A). The neutralizing antibody titers for subjects on Day 0, 28 schedule increased significantly 28 days after the second injection, when compared to those of subjects on Day 0,14 schedule within each dosage group. Almost similar trends like those observed for the neutralizing antibody were observed during the evaluation of the IgG antibody level (Fig. 2B). In addition, the neutralizing antibody titers significantly decreased with increasing age (Fig. 2C and 2D); younger subjects tended to have a higher level of neutralizing antibody titers .

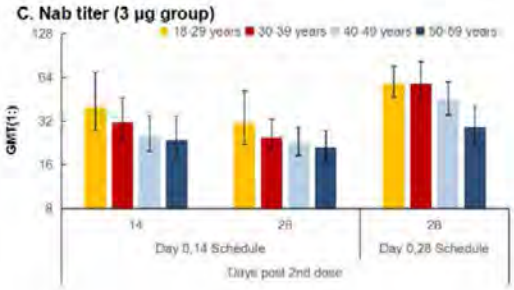
250



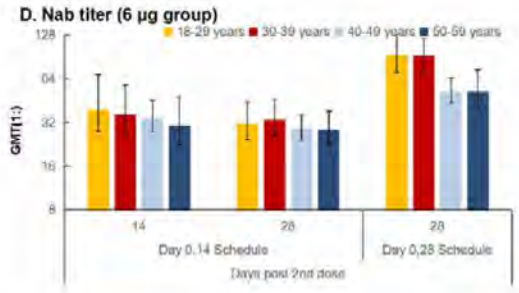
251



252



253



254 **Figure legends**

255 **Figure 2. Antibody Response in the Per-Protocol Cohort.**

(A) The neutralizing antibody titer in all participants 14 and 28 days after second dose in Day 0,14 schedule and 28 days after second dose in Day 0,28 schedule. (B) The RBD specific IgG antibody titer in all participants 14 and 28 days after second dose in Day 0,14 schedule and 28 days after second dose in Day 0,28 schedule. (C) The neutralizing antibody titer among different age-groups at different time points from all participants that received 3 μg vaccine. (D) The neutralizing antibody titer among different age-group at different time points from all participants that received 6 μg vaccine.

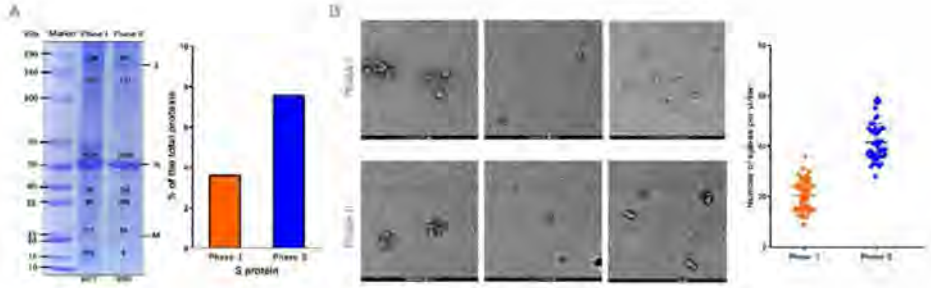


Figure legends

Figure 3. The proportion of Spikes in CoronaVac used for phase 1 and 2 vaccine evaluation.

(A) Protein composition analysis of CoronaVac samples from phase I and II by a NuPAGE 4-12% Bis-Tris gel, followed by whole-gel protein staining using Coomassie Blue gel staining reagent (45% methanol, 10% glacial acetic acid, 0.25% Coomassie Blue R-250). The viral protein bands of vaccine strain used for phase I and II were quantified by densitometry using ImageJ software with values depicted in the gel. The proportions of spikes to the total proteins in each gel lane in CoronaVac samples used forof phase 1 and 2 were calculated separately. (B) Representative negative staining images of the CoronaVac samples used in phase 1 and 2 trials. Three images were randomly selected for each phase. Grouped scatter plot showing the numbers of Spikes on two-dimensional projections of randomly selected 50 virions of CoronaVac samples used for phase I (left) and phase II (right), respectively.

277 **DISCUSSION**

278 This trial demonstrated that the 2 doses of different dosage of CoronaVac were well
279 tolerated and immunogenic in healthy adults aged 18-59 years. The incidence rates of
280 adverse reactions in the 6 μg and 3 μg group were comparable, indicating that there
281 was no dose-related aggravating concern on safety. Furthermore, no SAEs related to
282 vaccine occurred, and most adverse reactions reported were generally assessed to be
283 mild. The safety profile of CoronaVac is comparable to that observed in our phase 1
284 clinical trial [see the coordinated submission], and to other inactivated vaccine
285 formulations manufactured by Sinovac.[4,5] Compared with other COVID-19 vaccine
286 candidates, the incidence rate of fever was relatively low in our clinical trial, which
287 further indicates that CoronaVac was well tolerated.[6-10]

288 It's worth noting that the immune responses elicited in phase 2 were much better than
289 those recorded in phase 1, with seroconversion rates over 90%. Our preclinical
290 investigations had revealed that cell culture technology closely correlated with viral
291 propagation and affected viral morphology, protein composition and prefusion
292 conformation of spikes.[3] In both preclinical study and phase 1 trials, a 50-liter culture
293 of Vero cells grown in the Cell Factory system was used, while an optimized process
294 for growing cells using a highly automated bioreactor, where cell culture parameters
295 like dissolved oxygen, pH, and CO_2/O_2 gas levels, were controlled precisely, was
296 developed for producing the CoronaVac for phase 2 trial. To deduce the reasons
297 underlying the enhanced protective immune responses observed in phase 2 trial, we
298 examined the molecular differences between the CoronaVac used in phase 1 and 2
299 trials. Protein composition analysis of the purified inactivated SARS-CoV-2 virions
300 indicated that the bioreactor-produced CoronaVac possessed higher redundancy of

intact spike protein (~180 kDa) when compared to the Cell Factory-yielded CoronaVac (Fig. 3A). Quantitative analysis showed that the intact spike protein accounted for ~7% and ~ 3.7 of total protein mass used in phase 1 and 2 trials, respectively. Electron microscopic examination of the samples further verified that the average number of spikes per virion of the viral sample used in phase 2 trial was almost double to those used in phase 1 trial (Fig. 3B). These observations indicated that CoronaVac used in phase 2 trial contained more *bona fide* immunogens, which explains its better protective immune responses, highlighting the importance of developing an optimum manufacturing process and the integration of multiple-disciplinary techniques, such as genomics and structural biology to support a new era of precision vaccinology.

After two-dose vaccination, immune responses induced by Day 0,28 schedule was above the value of Day 0,14 schedule regardless of the dosage of the vaccine, which was consistent with our anticipation. By using Day 0,14 schedule, antibody response could be induced within a relatively short time period, and this schedule could be introduced to an emergency use and is of vital importance to handle COVID-19 pandemic situation. Regarding the Day 0,28 schedule, robust antibody response is generated and longer persistence could be expected, which supports the need for a routine use under the low incidence rate of COVID-19.

Nabs play an important role in virus clearance and have been considered as a key immune correlate for protection or treatment against viral diseases. Twenty-eight days after the two-dose vaccination, the Nab levels of individual schedules range from 23.8 to 65.4 in phase 2, which was lower than those of convalescent patients tested by the same method in the same laboratory, of which the Nab average level was 163.7.[11] We

assume the antibody level could provide satisfying protection against COVID-19 disease based on three reasons. Firstly, most of the surrogate endpoints based on neutralizing antibodies ranges from 8-24, such as EV71 and Varicella vaccines.[12,13] Secondly, experience from our preclinical study indicated that the neutralizing antibody titers of 1:24 elicited in macaques models conferred complete protection against SARS-CoV-2. Thirdly, several studies revealed that antibody responses generated from natural infection may decreased significantly, such as SARS-Cov-2, SARS-CoV and MERS-CoV,[14-16] however, recrudesce of these patients has been rarely reported, which indicated that the immunological memory might play an important role of prevention of re-infections.

Moreover, one prospective goal of our preclinical study and clinical trials was to establish a vaccine-induced surrogate of protection. Compared with vaccine inducing high level antibody, those inducing lower antibody level are more likely to produce evidence on surrogate of protection. Under above assumptions, the dosage of 3 μg with Day 0,14 or Day 0,28 schedule is adopted in our phase 3 trial.

When comparing antibody levels between age-groups, it should be noted that the neutralizing antibody titers significantly decreased with increasing age. These results are consistent with epidemiological trends observed in COVID-19 patients; those with moderate or severe symptoms tend to be elderly.[17] These results suggest that escalated dosage or extra dose of CoronaVac might be needed in elderly.

Several limitations of this trial should be noted. Firstly, we only assessed the humoral immunity in phase 2 trial, and more evaluation focus on response of Th1 and Th2 is ongoing. Secondly, we only reported immune response data on healthy adults, and do

not include data on more susceptible populations, such as elderly or with comorbidity; and also the immune persistence is not available yet, which need to be further studied. Thirdly, we didn't compare the neutralizing antibody titers induced by CoronaVac and convalescent COVID-19 patients in parallel, however, we conducted this detection of convalescent serum specimens with same procedure performed in this phase 2 trial.

In conclusion, favorable safety and immunogenicity of CoronaVac was demonstrated on both schedules and both dosages in this phase 2 clinical trial, which support the conduction of phase 3 trial with optimum schedule/dosage per different scenarios. Currently, our first priority is to evaluate the protective efficacy of the 3 μg dosage under Day 0,14 schedule. Moreover, Day 0,28 schedule with 3 μg vaccine will also be adopted in our future phase 3 clinical trials.

REFERENCES

1. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020;395:565-74.
2. World Health Organization. Draft landscape of COVID-19 candidate vaccines. July 14, 2020 (https://www .who .int/who -documents-detail/draft -landscape-of-covid-19-andidate-vaccines).
3. Gao Q, Bao L, Mao H, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science 2020;369:77-81.
4. Liu XE, Chen HY, Liao Z, et al. Comparison of immunogenicity between inactivated and live attenuated hepatitis A vaccines among young adults: a 3-year follow-up study. J Infect Dis 2015;212:1232-6.

5. Zhu F, Xu W, Xia J, et al. Efficacy, safety, and immunogenicity of an enterovirus 71 vaccine in China. N Engl J Med 2014;370:818-28.

6. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med 2020.

7. Zhu F-C, Li Y-H, Guan X-H, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet 2020;395:1845-54.

8. Zhu F-C, Guan X-H, Li Y-H, et al. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomized, double-blind, placebo-controlled, phase 2 trial. Lancet 2020.

9. Folegatti PM, Ewer KJ, Aley PK, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet 2020.

10. Mulligan MJ, Lyke KE, Kitchin N, et al. Phase 1/2 study to describe the safety and immunogenicity of a COVID-19 RNA vaccine candidate (BNT162b1) in adults 18 to 55 years of age: interim report. July 1, 2020 (https://wwwmedrxivorg/ content/10.1101/ 2020.06.30.20142570v1). preprint.

11. Wang X, Guo X, Xin Q, et al. Neutralizing antibodies responses to SARS-CoV-2 in COVID-19 inpatients and convalescent patients. Clin Infect Dis 2020.

12. Hao B, Chen Z, Zeng G, et al. Efficacy, safety and immunogenicity of live attenuated varicella vaccine in healthy children in China: double-blind, randomized, placebo-controlled clinical trial. Clin Microbiol Infect 2019;25:1026-31.

13. Jin PF, Li JX, Zhou Y, Zhu FC. Immunological surrogate endpoints to evaluate vaccine efficacy. Chin J Prev Med 2015;49:1110-4.

14. Seow J, Graham C, Merrick B, et al. Longitudinal evaluation and decline of antibody response in SARS-CoV-2 infection. July 11, 2020 (https://wwwmedrxivorg/content/10.1101/2020.07.09. 20148429). preprint.

15. Choe PG, Perera R, Park WB, et al. MERS-CoV antibody responses 1 year after symptom onset, South Korea, 2015. Emerg Infect Dis 2017;23:1079-84.

16. Cao W-C, Liu W, Zhang P-H, Zhang F, Richardus JH. Disappearance of antibodies to SARS-associated coronavirus after recovery. N Engl J Med 2007;357:1162-3.

17. Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020.

2. Combate as variantes do coronavírus

2.1. CoronaVac eleva imunidade contra variantes de quem já teve Covid-19, mostra estudo chinês

Um artigo publicado na revista Emerging Microbes and Infections demonstrou que, em indivíduos não vacinados e recuperados de Covid-19 há 12 meses, a imunização com a CoronaVac induziu alta atividade neutralizante contra as variantes delta, alfa e beta do vírus SARS-CoV-2, além de aumentar a quantidade de anticorpos. O estudo foi publicado no início do mês e conduzido por cientistas do Centro de Pesquisa de Doenças Infecciosas de Shenzhen, na China.

Participaram do estudo 22 pacientes convalescentes de Covid-19 que receberam uma vacina de vírus inativado um ano após a recuperação, sendo que 13 tomaram CoronaVac. Eles foram acompanhados durante três fases: (a) um mês depois da infecção, (b) um ano após a recuperação, antes da vacina, e (c) de duas semanas a três meses após a imunização.

Os cientistas avaliaram a duração da imunidade adquirida pela infecção e a eficácia da vacina. Decorridos 12 meses da recuperação, o nível de anticorpos IgG dos voluntários caiu 23,7%, e a capacidade de neutralização do vírus também foi reduzida, especialmente contra as variantes.

Com a administração da CoronaVac, houve um aumento de quatro vezes nos anticorpos IgG, atingindo níveis similares aos observados um mês após a infecção. Além disso, a atividade neutralizante contra a cepa original e contra as variantes aumentou de sete a 17 vezes.

"Os resultados apontam a queda de anticorpos neutralizantes um ano após a recuperação pela Covid-19, sugerindo um alto risco de reinfecção pelas novas cepas. A imunização com a vacina de vírus inativado potencializou a proteção tanto contra a cepa de Wuhan como contra as variantes", afirmam os autores no artigo.

Publicado em: 03/03/2022

Artigo

Emerging Microbes & Infections

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/temi20

The SARS-CoV-2 inactivated vaccine enhances the broad neutralization against variants in individuals recovered from COVID-19 up to one year

Xinrong Zhou, Lin Cheng, Haiyan Wang, Xuejiao Liao, Miao Wang, Lanlan Wei, Shuo Song, Bing Zhou, Zhenghua Ma, Huimin Guo, Xiangyang Ge, Bin Ju & Zheng Zhang

To cite this article: Xinrong Zhou, Lin Cheng, Haiyan Wang, Xuejiao Liao, Miao Wang, Lanlan Wei, Shuo Song, Bing Zhou, Zhenghua Ma, Huimin Guo, Xiangyang Ge, Bin Ju & Zheng Zhang (2022) The SARS-CoV-2 inactivated vaccine enhances the broad neutralization against variants in individuals recovered from COVID-19 up to one year, Emerging Microbes & Infections, 11:1, 753-756, DOI: 10.1080/22221751.2022.2043728

To link to this article: https://doi.org/10.1080/22221751.2022.2043728

Artigo

Emerging Microbes & Infections
2022, VOL. 11
https://doi.org/10.1080/22221751.2022.2043728

LETTER

OPEN ACCESS Check for updates

The SARS-CoV-2 inactivated vaccine enhances the broad neutralization against variants in individuals recovered from COVID-19 up to one year

Xinrong Zhou[a]*, Lin Cheng [a]*, Haiyan Wang[a]*, Xuejiao Liao[b]*, Miao Wang[a], Lanlan Wei[a], Shuo Song[a], Bing Zhou[a], Zhenghua Ma[b], Huimin Guo[a], Xiangyang Ge[a], Bin Ju[a,c] and Zheng Zhang[a,c,d]

[a]Institute for Hepatology, National Clinical Research Center for Infectious Disease, Shenzhen Third People's Hospital; The Second Affiliated Hospital, School of Medicine, Southern University of Science and Technology, Shenzhen, People's Republic of China; [b]Follow-up Department of Chronic Diseases, National Clinical Research Center for Infectious Disease, Shenzhen Third People's Hospital; The Second Affiliated Hospital, School of Medicine, Southern University of Science and Technology, Shenzhen, People's Republic of China; [c]Guangdong Key Laboratory for Anti-Infection Drug Quality Evaluation, Shenzhen, People's Republic of China; [d]Shenzhen Research Center for Communicable Disease Diagnosis and Treatment of Chinese Academy of Medical Science, Shenzhen, People's Republic of China

ARTICLE HISTORY Received 22 December 2021; Revised 7 February 2022; Accepted 14 February 2022

Since the outbreak of the coronavirus disease 2019 (COVID-19) in December 2019 [1], the continuously emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants have been identified and reported in different regions and countries worldwide, such as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Omicron (B.1.1.529), Kappa (B.1.617.1), Eta (B.1.525), and Iota (B.1.526) [2,3]. SARS-CoV-2 variants have contributed to several waves of the COVID-19 pandemic, whose transmissibility and infectivity are much higher than the original wild-type (WT) virus. More seriously, these variants largely escaped the neutralization by convalescent and vaccine-elicited plasma and monoclonal neutralizing antibodies (nAbs), greatly hindering the development of effective measures to prevent and control the virus infection.

Vaccination has long been a crucial measure to protect human against the infection of pathogens and can establish solid immune barriers in populations. Currently, various kinds of SARS-CoV-2 vaccines including inactivated vaccine (Sinopharm, Sinovac), DNA vaccine (Inovio), mRNA vaccine (Pfizer/BioNTech, Moderna), adenovirus vector vaccine (AstraZeneca/Oxford, Johnson & Johnson, Cansino), and protein vaccine (Novavax, Zhifei), showed good efficacy and therefore were adopted by various countries for population immunization [4–7]. For the individuals previously infected with SARS-CoV-2, it is debated whether they should be vaccinated or not. Indeed, the natural virus infection could induce robust antibody responses in COVID-19 patients which could be maintained after 7 months since the symptom onset [8]. However, the neutralizing activities of the convalescent plasma were gradually decreased, especially after one year of recovery [9], suggesting a risk of re-infection of SARS-CoV-2 variants.

Indeed, several studies have reported that breakthrough infections occurred in some vaccine recipients, indicating that there is a strong correlation between the immune protection and the value of nAbs [10]. Therefore, it is very important to monitor the longitudinal dynamics of plasma nAbs against the emerging SARS-CoV-2 variants continuously. The antibody responses to the mRNA and viral vector vaccines have been evaluated in individuals who previously infected with SARS-CoV-2 [11]. It is found that the high levels of nAbs against both the WT virus and variants were initialized by one or two doses of vaccines [12]. However, the antibody response to SARS-CoV-2 variants boosted by the inactivated vaccine in convalescent individuals is still unknown and the level of enhancement and the broad spectrum in neutralizing activity remain elusive.

In this study, we monitored the longitudinal dynamics of plasma IgG, IgA, and IgM binding to the SARS-CoV-2 WT receptor binding domain (RBD) in 22 of convalescent COVID-19 individuals who received at least one dose of inactivated vaccine (Figure S1A, Table S1). The levels of RBD-specific antibodies gradually declined with time over. Then we evaluated the changes of plasma antibodies after the inoculation of the inactivated vaccine. The follow-up period was divided into three phases including the early stage of recovery (median time: one month), late stage of recovery (median time: one year, i.e.

CONTACT Bin Ju jubin2013@163.com; Zheng Zhang zhangzheng1975@aliyun.com
*These authors contributed equally to this work.
Supplemental data for this article can be accessed at https://doi.org/10.1080/22221751.2022.2043728

before vaccination), and post vaccination (Figure S1B). The geometric mean values of RBD-specific IgG, IgA, and IgM decreased to 23.7%, 34.4%, and 29.1%, respectively in the late stage of recovery (Figure S1C). After the vaccination with inactivated vaccine, the levels of IgG had 3.9-fold increase as compared with those before vaccination, and reached the similar levels with those in the early stage of recovery. By contrast, the boosted vaccination failed to induce a recalling IgA or IgM response, suggesting that virus-specific IgG may play a more important role in the long-term antibody protection.

To further evaluate the neutralizing activities of plasma against SARS-CoV-2 variants, we constructed seven kinds of pseudoviruses based on the HIV-1 backbone, including WT (Wuhan reference strain), Alpha, Beta, Delta, Kappa, Eta, and Iota variants (Figure S2), and then performed the pseudovirus-neutralization assay. The diverse mutations in the region of spike protein contributed to their distinct neutralizing resistance. As shown in Figure 1(a), Figure S3, and Table S2, although the convalescent plasma showed effective neutralizing activities in the early stage of recovery, their geometric mean titers (GMTs) of

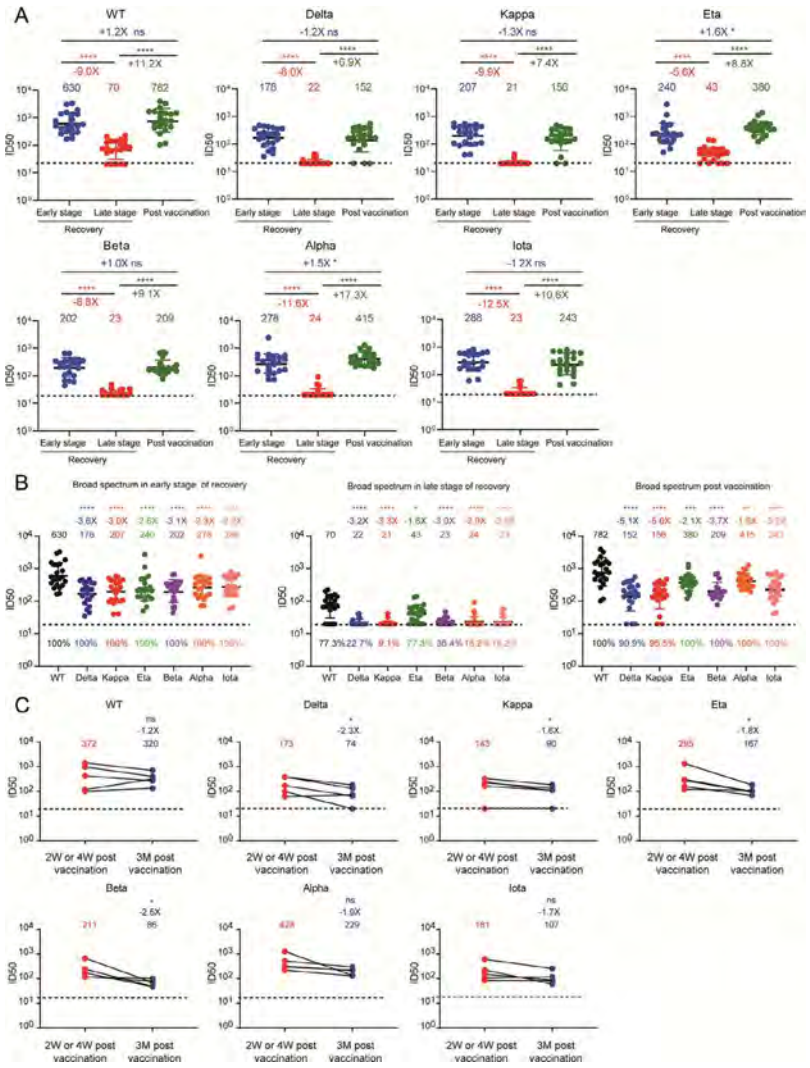


Figure 1. Neutralizing activities against WT SARS-CoV-2 and variants in convalescent individuals received the inactivated vaccine. (a) Plasma neutralizing activities against each SARS-CoV-2 strain in three follow-up time points were measured and shown in the values of 50% inhibition dilution (ID_{50}). (b) The broad spectrum of plasma nAbs in three follow-up time points. The GMT of nAbs against each variant was compared to that against WT, respectively. The positive rates of nAbs were marked at the bottom of each column. (c) The durability of broadly nAbs post the boosted vaccination. Paired plasma samples were collected from five individuals at Week 2 ($n = 4$) or Week 4 ($n = 1$) and Month 3 post vaccination and then tested the neutralizing activities against WT SARS-CoV-2 and variants. "-" represents decreased neutralization activity, "+" represents increased neutralization activity. The paired *t* test is performed here. "****" means $P < .0001$, "***" means $P < .001$, "**" means $P < .01$, "*" means $P < .05$, "ns" means not significant. The GMT, fold-change, and significance of difference were labeled on the top. The limit of detection was 1:20 dilution. The data below the limit was set to 20 for visualization.

nAbs were largely decreased in the late stage of recovery, especially against various SARS-CoV-2 variants. After the boosted vaccination with inactivated vaccine, the neutralizing activities of plasma against the WT and six mutated viruses we tested were significantly increased 6.9-fold to 17.3-fold as compared with those before vaccination, whose levels were compatible to those in the early stage of recovery.

To better evaluate the broadness of plasma nAbs in different stages of follow-up, we rearranged these neutralization results by different time periods to directly compare their neutralizing values against SARS-CoV-2 variants. As shown in Figure 1(b), the plasma collected in the early stage of recovery had high levels of nAbs against both WT SARS-CoV-2 and variants, whose GMTs ranged from 176 to 630 and neutralizing antibody positive rates were 100% in all seven tested viruses. Along with the time over, the levels of nAbs were significantly decreased after one year. Especially, most of plasma samples lost their neutralizing activities against SARS-CoV-2 variants, and the positive rates of nAbs had also been dropped to 9.1% to 77.3%. Among them, a total of 22 individuals accepted at least one dose of inactivated vaccine and contributed their blood samples. The levels of nAbs were remarkably increased as compared with those before vaccination and rapidly raised to the similar levels in the early stage of recovery. Meanwhile, the positive rates of nAbs against SARS-CoV-2 variants were also increased to 90.9% to 100%. Thus, we clearly demonstrated that the vaccination with inactivated vaccine rapidly enhanced the neutralizing activities against the SARS-CoV-2 variants in individuals who have recovered from COVID-19 up to one year.

Finally, we also explored the durability of neutralizing antibody response elicited by the boosted vaccination in these convalescent individuals. We have obtained serial plasma samples from five individuals (donor 2, 11, 16, 19 and 20) at Week 2 or Week 4 and Month 3 post vaccination. As shown in Figure 1 (c) and Figure S4, the levels of nAbs were slightly decreased with time, but dropped more obviously against the variants including Delta, Kappa, Eta, and Beta. These results suggested that it is very important to monitor the levels of nAbs against the emerging SARS-CoV-2 variants in the convalescent COVID-19 individuals.

Compared with several previous studies, the results described here were rational and novel. Xiang et al detected the levels of nAbs against Beta variant in convalescent patients one year after infection, and found that those individuals who effectively neutralized the WT virus displayed limited neutralizing activities against Beta variant (diminished to 22.6%) [13]. Furthermore, Li et al detected the RBD-specific antibody responses in 1782 plasma samples from 869 convalescent donors after 12 months post infection in Wuhan, China, and found that the levels of plasma IgG and nAbs significantly declined with time [9]. Combined with our study, these results emphasized the risk of reinfection with SARS-CoV-2 variants in convalescent COVID-19 individuals recovered more than one year. Since the different vaccines have diverse immunogenicity, the effectiveness of all being applied SARS-CoV-2 vaccines should be comprehensively evaluated. Lucas et al had analyzed the immune response to SARS-CoV-2 in the cohorts of previously infected (recovered) or uninfected (naive) individuals who received the mRNA vaccine. The results showed that individuals in both groups obtained neutralization capacity against all tested variants. Moreover, plasma samples from previously infected individuals exhibited better neutralizing activities than those from uninfected donors generally. After the vaccination with mRNA vaccine, the high levels of nAbs could persist about 4–6 months, and were then reduced over time because of the waning immunity [14]. In addition, similar immune responses were also observed in the population who previously infected with SARS-CoV-2 and then received one dose of Ad26.COV2.S vaccine or the replicating poxvirus vector-based RBD vaccine, suggesting that the boosted vaccination could bring a solid immune protection to the convalescent individuals. However, it lacks the report about the antibody responses in convalescent individuals after the boosted vaccination with the inactivated vaccine. Our results here demonstrated that the vaccination with inactivated vaccine was also effective in enhancing the levels of nAbs in convalescent individuals, especially against the emerging SARS-CoV-2 variants. Importantly, according to a recent research report, the titers of nAbs were positive correlation with immune efficacy against COVID-19. The vaccinators with ID_{50} values of 10, 100, and 1000 owned 78%, 91%, and 96% immune efficacy, respectively, after 4 weeks inoculated with mRNA vaccine [15], suggesting that the convalescent patients obtained high immune efficacy in the early stage of recovery and post the vaccination with inactivated vaccine in this study.

In conclusion, we evaluated the plasma neutralization against various SARS-CoV-2 variants in the convalescent individuals who received the inactivated vaccine. These results showed that the levels of broadly nAbs were significantly decreased in the convalescent individuals after one year since they recovered from COVID-19, suggesting the high risk of reinfection of various emerging variants. The vaccination with inactivated vaccine potentially improved the plasma neutralizing activities against the WT SARS-CoV-2 and variants, which could even last for 3 months post vaccination. This study for the first time demonstrated that the inactivated vaccine potentially induced the

neutralizing activity against the emerging SARS-CoV-2 variants in the convalescent individuals, which could minimize the risk of breakthrough infections in future.

Acknowledgments

We thank all participants who recovered from COVID-19 and all of the healthy providers from Shenzhen Third People's Hospital for the work they have done.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by the National Science Fund for Distinguished Young Scholars (82025022), the National Natural Science Foundation of China (82002140, 82171752, 82101861), the Guangdong Basic and Applied Basic Research Foundation (2021B1515020034, 2019A1515011197, 2021A1515011009, 2020A1515110656), and the Shenzhen Science and Technology Program (RCYX20200714114700046, JSGG20200207155251653, JCYJ20210324131606018, JSGG20200807171401008, KQTD20200909113758004, JCYJ20190809115617365).

Data availability statements

We are happy to share reagents and information in this study upon request.

ORCID

Lin Cheng http://orcid.org/0000-0001-8066-527X

References

[1] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382:727–733.
[2] Mlcochova P, Kemp S, Dhar MS, et al. SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. Nature. 2021;599:114–119.
[3] Zhang L, Li Q, Liang Z, et al. The significant immune escape of pseudotyped SARS-CoV-2 variant omicron. Emerg Microbes Infect. 2022;11:1–5.
[4] Jara A, Undurraga EA, Gonzalez C, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. N Engl J Med. 2021;385:875–884.
[5] Voysey M, Clemens S, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomized controlled trials in Brazil, South Africa, and the UK. Lancet. 2021;397:99–111.
[6] Xia S, Zhang Y, Wang Y, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomized, double-blind, placebo-controlled, phase 1/2 trial. Lancet Infect Dis. 2021;21:39–51.
[7] Subbarao K. The success of SARS-CoV-2 vaccines and challenges ahead. Cell Host Microbe. 2021;29:1111–1123.
[8] Ju B, Zhang Q, Ge J, et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection. Nature. 2020;584:115–119.
[9] Li C, Yu D, Liang H, et al. Twelve-month specific IgG response to SARS-CoV-2 receptor-binding domain among COVID-19 convalescent plasma donors in wuhan. Nat Commun. 2021;12:4846.
[10] Vignier N, Berot V, Bonnave N, et al. Breakthrough infections of SARS-CoV-2 Gamma variant in fully vaccinated gold miners, French guiana, 2021. Emerg Infect Dis. 2021;27:2673–2676.
[11] Keeton R, Richardson S, Moyo-Gwete T, et al. Prior infection with SARS-CoV-2 boosts and broadens Ad26.COV2.S immunogenicity in a variant-dependent manner. Cell Host Microbe. 2021;29:1611–1619.
[12] Urbanowicz RA, Tsoleridis T, Jackson HJ, et al. Two doses of the SARS-CoV-2 BNT162b2 vaccine enhance antibody responses to variants in individuals with prior SARS-CoV-2 infection. Sci Transl Med. 2021;13:j847.
[13] Xiang T, Liang B, Fang Y, et al. Declining levels of neutralizing antibodies against SARS-CoV-2 in convalescent COVID-19 patients one year post symptom onset. Front Immunol. 2021;12:708523.
[14] Lucas C, Vogels CBF, Yildirim I, et al. Impact of circulating SARS-CoV-2 variants on mRNA vaccine-induced immunity. Nature. 2021;600(7889):523–529.
[15] Gilbert PB, Montefiori DC, McDermott AB, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. Science. 2021: eab3435.

2.2. Dose de reforço da CoronaVac é eficaz contra a ômicron, aponta estudo chileno

Um estudo clínico de fase 3 publicado na plataforma MedRxiv mostrou que a terceira dose da CoronaVac, vacina do Butantan e da Sinovac, aumenta significativamente a resposta de células T e a quantidade de anticorpos neutralizantes capazes de reconhecer as variantes ômicron e delta do SARS-CoV-2. A pesquisa foi coordenada pela Pontifícia Universidade Católica do Chile e pelo Instituto Milênio de Imunologia e Imunoterapia, que já tinham divulgado resultados preliminares do estudo em dezembro de 2021.

Os pesquisadores incluíram no estudo 186 voluntários que receberam a dose de reforço da vacina do Butantan após seis a oito meses da segunda dose. Nesse período, o nível de anticorpos neutralizantes, que estava em 124,8 GMU (unidades médias geométricas) um mês após a segunda dose, foi reduzindo até 39 GMU – fator que também foi observado em outras vacinas, justificando a necessidade de um reforço.

Com a terceira dose da CoronaVac, os anticorpos neutralizantes saltaram para 499 GMU, um aumento de 12 vezes. Ao analisar a capacidade de neutralização contra a ômicron e a delta em 30 dos voluntários, os cientistas identificaram títulos médios de anticorpos neutralizantes de 50,7 contra a ômicron e de 159,2 contra a delta, e a taxa de soropositividade (produção de anticorpos) foi de 76,7% e 93%, respectivamente.

A resposta imune celular, mais especificamente a atividade de células T CD4+, foi avaliada em 40 dos participantes. "Nós observamos que a ativação das células T se manteve alta seis meses após a segunda dose e seguiu aumentando após a dose de reforço, sugerindo que a CoronaVac pode estimular e sustentar a resposta imune celular ao longo do tempo", afirmam os autores do estudo. As células T também apresentaram uma boa resposta à ômicron e à delta, semelhante à resposta contra a cepa original do SARS-CoV-2.

Em análise divulgada em outubro de 2021 pelo Ministério da Saúde do Chile, a proteção da vacina contra hospitalizações pela Covid-19 no país aumentou de 84% para 88% após a dose de reforço. Outros estudos já mostraram que a dose de reforço da CoronaVac potencializa a resposta imune, inclusive contra variantes do coronavírus.

Publicado em: 07/02/2022

Artigo

Emerging Microbes & Infections

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/temi20

The SARS-CoV-2 inactivated vaccine enhances the broad neutralization against variants in individuals recovered from COVID-19 up to one year

Xinrong Zhou, Lin Cheng, Haiyan Wang, Xuejiao Liao, Miao Wang, Lanlan Wei, Shuo Song, Bing Zhou, Zhenghua Ma, Huimin Guo, Xiangyang Ge, Bin Ju & Zheng Zhang

To cite this article: Xinrong Zhou, Lin Cheng, Haiyan Wang, Xuejiao Liao, Miao Wang, Lanlan Wei, Shuo Song, Bing Zhou, Zhenghua Ma, Huimin Guo, Xiangyang Ge, Bin Ju & Zheng Zhang (2022) The SARS-CoV-2 inactivated vaccine enhances the broad neutralization against variants in individuals recovered from COVID-19 up to one year, Emerging Microbes & Infections, 11:1, 753-756, DOI: 10.1080/22221751.2022.2043728

To link to this article: https://doi.org/10.1080/22221751.2022.2043728

Pública de Chile. [6]Laboratorio de Virología Molecular y Celular, Programa de Virología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile. [7]Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology (LJI), La Jolla, CA 92037, USA. [8]Department of Medicine, Division of Infectious Diseases and Global Public Health, University of California, San Diego (UCSD), La Jolla, CA 92037, USA. [9]Sinovac Biotech, Beijing, China. [10]Departamento de Farmacia, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile. [11]Departamento de Endocrinología, Facultad de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.

[#] These authors contributed equally to this work.

*Corresponding authors.

Keywords:

CoronaVac; Phase 3 clinical trial; SARS-CoV-2; COVID-19; inactivated vaccine, Booster dose.

Abstract

Background: CoronaVac® is an inactivated SARS-CoV-2 vaccine approved by the World Health Organization. Previous studies reported increased levels of neutralizing antibodies and specific T cells two- and four-weeks after two doses of CoronaVac®, but the levels of neutralizing antibodies are reduced at six to eight months after two doses. Here we report the effect of a booster dose of CoronaVac® on the anti-SARS-CoV-2 immune response generated against variants of concern (VOC) Delta and Omicron in adults participating in a phase 3 clinical trial in Chile.

Methods: Volunteers immunized with two doses of CoronaVac® in a four-week interval received a booster dose of the same vaccine between twenty-four and thirty weeks after the 2nd dose. Four weeks after the booster dose, neutralizing antibodies and T cell responses were measured. Neutralization capacities and T cell activation against VOC Delta and Omicron were detected at four weeks after the booster dose. **Findings**: We observed a significant increase in neutralizing antibodies at four weeks after the booster dose. We also observed an increase in $CD4^+$ T cells numbers over time, reaching a peak at four weeks after the booster dose. Furthermore, neutralizing antibodies and SARS-CoV-2 specific T cells induced by the booster showed activity against VOC Delta and Omicron. **Interpretation**: Our results show that a booster dose of CoronaVac® increases the anti-SARS-CoV-2 humoral and cellular immune responses in adults. Immunity induced by a booster dose of CoronaVac® is active against VOC, suggesting an effective protection.

Background

The ongoing pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has promoted the rapid development of safe, immunogenic, and effective vaccines against SARS-CoV-2 to be used by the general population, which have successfully reduced the transmission of the disease burden. CoronaVac® is an inactivated SARS-CoV-2 vaccine developed by Sinovac Life Sciences Co., Ltd. (Beijing, China) and is among the current vaccines approved by the World Health Organization (WHO) to combat coronavirus disease 2019 (COVID-19) and one of the most used vaccines worldwide[1,2]. Phase I/II clinical trials in China demonstrated that this vaccine induces cellular and humoral response upon immunization [3–5]. Furthermore, the ongoing phase 3 clinical trial in Chile has described an increase in the levels of IgG and neutralizing antibodies in adults aged 18-59 years and ≥ 60 years two- and four-weeks after the second dose of CoronaVac® [56]. In addition, this vaccination promotes the activation of a T cell immune response against SARS-CoV-2 antigens in a 0-14 immunization schedule [5] (two-weeks interval), being an effective vaccine to prevent COVID-19 [7,8]. In Chile, 93.7% of the target population has received a first vaccine dose, and 91.4% were fully vaccinated with CoronaVac® on December 10th of 2021 in a 0-28 days vaccination schedule [9]. Although this primary immunization schedule induces neutralizing antibody present in the serum of vaccinated people [10], these titers decrease in time [6,11,12] and have lower levels of neutralization against highly transmissible variants of concern (VOC) as compared to the original vaccine strain, potentially decreasing the effectiveness of these vaccines as new variants emerge

[13–17]. For these reasons, the use of booster doses was approved in Chile in August 2021 in Chile for high-risk populations and adults at five months after administration of the second dose [18]. In this sense, a report published in October 2021 in Chile, showed that the effectiveness of CoronaVac® against COVID-19 increase from 56% to 80% fourteen days after the application of the booster dose [19]. Notably, a previous study performed in adults aged 18-59 years old demonstrated that a booster dose of CoronaVac®, applied six months after the first dose to individuals that previously received two doses of this vaccine, increased the levels of antibodies 3-5-fold as compared to the levels observed four weeks after the second dose [12]. Here, we further extend these findings by reporting the levels of neutralizing antibodies and specific T cells against SARS-CoV-2 and its activity against VOC Delta and Omicron in adults ≥18 years old that participated in the phase 3 clinical trial carried out in Chile, who were vaccinated in a 0-28-days vaccination schedule and received a booster dose five months after the second dose.

Materials and methods

Volunteers and sample collection

Blood samples were obtained from volunteers recruited in the clinical trial CoronaVac03CL (clinicaltrials.gov #NCT04651790) carried out in Chile starting in November 2020. The Institutional Scientific Ethical Committee of Health Sciences reviewed and approved the study protocol at the Pontificia Universidad Católica de Chile (#200708006). Trial execution was approved by the Chilean Public Health Institute (#24204/20) and was conducted according to the current Tripartite

Guidelines for Good Clinical Practices, the Declaration of Helsinki [20], and local regulations. Informed consent was obtained from all volunteers upon enrollment. Volunteers receive two doses of CoronaVac® (3 μg or 600SU of inactivated SARS-CoV-2 inactivated along with alum adjuvant) in a four-week interval (0-28-day immunization schedule) and then a booster dose five months after the second dose. A complete inclusion and exclusion criteria list have been reported [5]. On November 11th, 2021, one hundred and eighty-six volunteers in the immunogenicity branch received the booster dose. The antibody and cell mediated immune responses were evaluated volunteers that had completed all their previous visits in one of the centers of the study (**Figure 1A**). Blood samples were obtained from all the volunteers before administration of the first dose (pre-immune), two, four, and twenty weeks (or five months) after the second dose, and four weeks after the booster dose (**Figure 1B**).

Procedures

The presence of antibodies against RBD with neutralizing capacities were measured in sera from seventy-seven volunteers that had completed all their study visits, including one month after the booster dose of CoronaVac®. The neutralizing capacities of circulating antibodies were evaluated by a surrogate virus neutralization test (sVNT) (Genscript Cat#L00847-A). Samples were two-fold serially diluted starting at a 4-fold dilution until reaching a 512-fold dilution. Assays were performed according to the instructions of the manufacturer and as reported previously [5]. Neutralizing antibody titers were determined as the last fold dilution in which the interaction between hACE2 and RBD was inhibited by 30% or more. Samples with a percentage of inhibition ≤30% at the lowest dilution (1:4) were

assigned as seronegative with a titer of 2. A sample was considered seropositive when its titer was higher than the pre-immune titer. The percentage of inhibition was determined as: 100 * [OD450nm value of negative control - OD450nm value of sample] / [OD450nm of negative control]. A standard curve was used to plot the neutralization response in the samples as international units (IU) using the WHO International Standard for SARS-CoV-2 antibody (NIBSC code 20/136), which was prepared according to the instructions of the manufacturer [21]. Data were analyzed using a sigmoidal curve model with a logarithm transformation of the concentration, and the final concentration for each sample was the average of the product of the interpolated IU from the standard curve and the sample dilution factor required to reach the OD450 value that falls within the linear range determined for each sample. Samples with undetermined concentration at the lowest dilution tested (1:4) were assigned to the lower limit of quantification (16.4 IU). The Geometric Mean Units (GMU) and titers (GMT) were represented in **Figure 2** and **Supplementary Figure 1,** respectively**. Table 2** shows comparisons among the visits.

Conventional virus neutralization tests (cVNT) were performed in sixty-two of the previous seventy-seven volunteers and evaluated as previously reported [5]. Briefly, Vero E6 cells were infected with a SARS-CoV-2 strain obtained by viral isolation in tissue cultures (33782CL-SARS-CoV-2 strain, D614G variant). Neutralization assays were carried out by the reduction of cytopathic effect (CPE) in Vero E6 cells (ATCC CRL-1586). The titer of neutralizing antibodies was defined as the highest serum dilution that neutralized virus infection, at which the CPE was absent as compared with the virus control wells (cells with CPE). Vero E6 cells

were seeded in 96-well plates (4×10^4 cells/well). For neutralization assays, 100 µL of 33782CL-SARS-CoV-2 (at a dose of 100 $TCID_{50}$) were incubated with serial dilutions of heat-inactivated sera samples (dilutions of 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, and 1:512) from participants for 1h at 37 °C. Cytopathic effect on Vero E6 cells was analyzed 7 days after infection.

A pseudotyped virus neutralization test (pVNT) assay was performed to assess the capacity of the antibodies against SARS-CoV-2 VOC in samples from thirty volunteers of the seventy-seven previously analyzed by sVNT. As previously reported [14], a HIV-1 backbone expressing firefly luciferase as a reporter gene and pseudotyped with the SARS-CoV-2 spike glycoproteins (HIV-1-SΔ19) from from lineage B.1 (D614G) or variants Delta (T19R, del157/158, L452R, T478K, D614G, P681R, D950N) and Omicron (A67V, ΔH69-V70, T95I, Y145D, ΔG142 -V143-Y144, ΔN211, EPE 213-214, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, T547K, D614G, H655Y, N679K, P681H, N764K, N865K, Q954H, N969K, L981F) was prepared as previously described [22]. Serum samples were two-fold diluted starting at 1:10 or 1:4 and the estimation of the ID80 was obtained using a 4-parameter nonlinear regression curve fit measured as the percent of neutralization determined by the difference in average relative light units (RLU) between test samples and pseudotyped virus controls. Also, cVNT assays were performed to assess the capacity of the antibodies against SARS-CoV-2 Delta variant in samples from nineteen volunteers of the seventy-seven previously analyzed by sVNT.

ELISPOT and flow cytometry assays were performed to evaluate the cellular immune response in forty volunteers of the seventy-seven previously analyzed

(**Figure 1A**), stimulating PBMCs with four Mega Pools (MPs) of peptides derived from the proteome of SARS-CoV-2 [23]: peptides from the S protein of SARS-CoV-2 (MP-S), the remaining proteins of the viral particle (excluding S protein peptides) (MP-R), and shorter peptides from the whole proteome of SARS-CoV-2 (MP-CD8-A and MP-CD8-B) [23]. Thirty of the previously analyzed volunteers were also stimulated with three Mega Pools (MP) of VOC, provided by La Jolla Institute for Immunology. MP derived from the S protein of SARS-CoV-2 WT, SARS-CoV-2 B1.617.2 MP 4326 (Delta variant), and SARS-CoV-2 B1.1.529 MP 4359 (Omicron variant) [14] were used to evaluate T cell activation at four weeks after the booster dose. Positive and negative controls were held for each assay. The number of Spot Forming Cells (SFC) for interferon gamma (IFN-γ) were determined by ELISPOT and the expression of Activation-Induced Markers (AIM) by T cells was evaluated by flow cytometry. Assays were performed according to the instructions of the manufacturer and as reported previously [5]. Further details on the ELISPOT assay, antibodies used for flow cytometry, and the respective protocols can be found in the Supplementary information and **Supplementary Table 1**.

Interleukin 2 (IL-2) and IFN-γ secretion were evaluated in the supernatants of twenty-two volunteers previously stimulated for 20h with SARS-CoV-2 MP of peptides derived from the Spike protein of VOC, using a Luminex 200 xMap multiplex system (Luminex Corporation, Austin, TX). The limit of detection for the cytokine measured ranged from 4.2 to 13,390 pg/mL, according to manufacturer's instructions. Further experimental details can be found in **Supplementary Information**.

207

208 **Statistical analyses**

209 Statistical differences for the immunogenicity results considered repeated
210 measures ANOVA with the Geisser-Greenhouse correction and Dunnet's *a*
211 *posteriori* multiple tests to compare between the booster dose and the other visits.
212 Analyses were performed over the base 10 logarithms of the data for neutralizing
213 antibody by sVNT, cVNT and pVNT. Cellular immune responses were analyzed by
214 a Friedman test for repeated measures for ELISPOT and flow cytometry for the
215 comparisons between booster dose and the other visits. Secretion of cytokines
216 were compared between the secretion induced by the WT strain against the VOC
217 Delta and Omicron by repeated measures ANOVA. The significance level was set
218 at 0.05 for all the analyses. All data were analyzed with GraphPad Prism 9.0.1.

219

220 **Results**

221 **A booster dose of CoronaVac® induces a significant increase in**
222 **antibody titers with neutralizing capacity in adults.**

223 One hundred and eighty-six volunteers from the immunogenicity branch that
224 received a booster dose of the CoronaVac® were included in this study. The first
225 dose of the vaccine was inoculated from January to March of 2021, and the second
226 dose was inoculated 28 days after the first one. The neutralizing capacity of serum
227 antibodies was evaluated in seventy-seven and sixty-two volunteers by sVNT and
228 cVNT, respectively, at the five different time-points indicated in **Figure 1B**.

229 As shown in **Figures 2A and D**, the peak level of antibodies with
230 neutralizing capacity in the total population evaluated, tested by sVNT and cVNT,

231 is reached at two weeks after the second dose (GMU 168.0, 95% CI=19.5-34.2
232 and GMT, 95% CI=) and four weeks after the second dose (GMU 124.8, 95%
233 CI=96.3-161.7 and GMT 13.5, 95% CI=9.6-19.2). However, this neutralizing
234 capacity significantly decreased twenty weeks after the second dose (GMU 39.0,
235 95% CI=32.4-47.0 and GMT 8.3, 95% CI=9.6-19.2), which is in line with previous
236 reports where the immunity against SARS-CoV-2 wanes six months after infection
237 or vaccination [24,25]. After the booster dose, the neutralizing capacity of the
238 antibodies increased even more than the one reported two weeks after the second
239 dose (GMU 499.0, 95% CI=370.6-673.0 and GMT 89.5 ± 64.0-125.2). Overall, we
240 observed that four weeks after the booster dose the neutralizing capacity increased
241 more than 12-fold (sVNT) and 10-fold (cVNT) as compared to the response at
242 twenty weeks after the second dose, and almost 3-fold as compared to two weeks
243 after the second dose (**Figures 2A and D**).

244 In adults 18-59 years old, the neutralizing capacity of circulating antibodies
245 tested by sVNT and cVNT (**Figures 2B and E**, respectively) reached its maximum
246 four weeks after the booster dose (GMU 918.8, 95% CI=623.4-1354 and 176.9,
247 95% CI=111.7-280.1) increasing more than 18- and 12-fold as compared to five
248 months after the second dose (GMU 48.9, 95% CI=37.6-63.5 and GMT 14.2, 95%
249 CI=7.1-28.4) and more than 4-fold as compared to two weeks after the second
250 dose (GMU 220.2, 95% CI=150.7-321.7 and GMT 17.5, 95% CI=9.8-31.3)
251 (**Figures 2B and E**). The seropositivity rate in this group reached 100% at four
252 weeks after the booster dose (**Table 2**). On the other hand, 53.2% of the total
253 volunteers analyzed here were adults ≥60 years. In this group, the same tendency

was observed, as seen in **Figure 2C and F**, observing an increase in the level of neutralizing antibodies evaluated by both techniques of more than 9-fold at four weeks after the booster dose (GMU 300.5, 95% CI=203.5-443.6 and GMT 47.3, 95% CI=32.1-69.5) as compared to the response observed twenty weeks after the second dose (GMU 32.4, 95% CI=25.1-41.8 and GMT 5.0, 95% CI=3.5-7.0). Equivalent to the 18-59 years old group, the seropositivity rate in this age group reached 100% four weeks after the booster dose (**Table 2**). The seropositivity rate achieved at four weeks after the booster dose was the highest when compared with the other visits in this study in the total vaccinated group and in both groups analyzed.

A booster dose of CoronaVac® induces a robust cellular immune response in adults.

The cellular responses following a booster dose of CoronaVac® were evaluated in 40 volunteers. We observed that $CD4^+$ T cell activation was increased twenty weeks after the second dose as compared to the other time points in both age groups, suggesting that CoronaVac® can stimulate $CD4^+$ T cell responses that are sustained over time (**Figure 3A-C**). Importantly, we observed a significantly further increase in $CD4^+$ T cell activation in both age groups following the booster dose, as compared to the pre-immune sample and samples obtained at two and four weeks after the second dose (**Figure 3A-C**). However, this difference was not significant as compared to the sample obtained twenty weeks after the second dose (**Figure 3A-C)**. Moreover, we did not observe a significant increase in the expression of AIM by $CD8^+$ T cells following the booster dose as compared to any

other time point, suggesting that specific $CD8^+$ T cell responses induced by CoronaVac® are not detected with the current methodologies, even after a third dose (**Supp. Figure 2A and C**). Accordingly, we observed an increase in IFN-γ production upon stimulation with mega-pools of peptides (MPs) S and R by ELISPOT at four weeks after the booster dose for both groups, as compared to the pre-immune sample (**Figure 3D-F**). As in the case of flow cytometry, we did not observe significant increase of IFN-γ^+ SFCs upon stimulation with CD8 MPs at any time point (**Supp. Figure 2**). These results suggest that although humoral responses decrease over time following vaccination with CoronaVac®, $CD4^+$ T cell responses remain significantly increased as compared to pre-immune samples and the booster dose promotes a small increase both IFN-γ production and $CD4^+$ T cell activation that is not significantly different as compared to the levels observed 20 weeks after the second dose.

Neutralizing antibodies and specific T cells induced by a booster dose of CoronaVac® recognize Delta and Omicron variants of SARS-CoV-2.

As we observed that the neutralization capacity and the T cell responses increased significantly with the booster dose and knowing that vaccinated volunteers exhibit decreased neutralization against VOC[14], we proceeded to evaluate the neutralizing capacities of antibodies in the serum from thirty booster-vaccinated individuals in pseudotyped virus neutralization test (pVNT) assay against two variants of concern of SARS-CoV-2, comparing with the level obtained for the D614G SARS-CoV-2 variant (B.1 lineage, **Figure 4A-B**). We observed that

the titers of antibodies with neutralizing capacities against Delta and Omicron variant show a significant reduction as compared to the levels achieved for the D61G variant (D614G: GMT 241.8, CI=155.7-375.6, Delta: 159.2, CI=99.1-256.0 and Omicron: GMT 50.7, CI=30.4-84.8), with a reduction of 1.5 for Delta and 4.8 for Omicron (**Figure 4A**). However, when we compared the changes in seropositivity for Delta and Omicron (**Figure 4B**), we observed a 93% and 76.7%, respectively, following the booster dose (**Table 3**). Neutralization assays against Delta variant with a cVNT in a different group of nineteen volunteers also show that antibodies induced four weeks after the booster dose have reduced capacity to neutralize this VOC (**Supp Figure 4A**), although the seropositivity rate observed is 84% (**Supp Figure 4B**).

The cellular responses for VOC following a booster dose of CoronaVac® were also evaluated in thirty volunteers using MPs of peptides derived from the Spike protein of Delta and Omicron variants. We observed equivalent numbers of AIM by $CD4^+$ T cells after four weeks of the booster dose upon stimulation with MP-S of SARS-CoV-2 WT, Delta, or Omicron variant (**Figure 5A**), with no significant differences between the response against the MP-S of the variants as compared to the MP-S of the WT strain. IFN-γ secreting T cells were also analyzed in these samples and no differences were observed **(Figure 5B)**. We also quantified the production of different cytokines in the supernatant of PBMCs stimulated with the MP-s of WT, Delta, and Omicron variants, observing that at four weeks after the booster dose the stimulated cells secrete equivalent levels of IL-2 and IFN-γ (**Figure Supp 3**). These results suggest that although the humoral response

measured as neutralization capacities and seroconversion against these VOC is lower as compared to the humoral response against the D614G strain, the cellular responses against SARS-CoV-2 VOC is equivalent to the responses elicited by the wild type strain in volunteers vaccinated with booster dose.

Discussion

In this report we evaluated the humoral and cellular immune response generated four weeks after the application of a booster dose of inactivated CoronaVac® vaccine in a cohort of volunteers enrolled in the phase 3 clinical trial held in Chile. The data reported here showed that although there was an adequate humoral response after two doses of CoronaVac®, with a 65.9% of effectiveness in preventing COVID-19 [8], both the sVNT and cVNT assays showed a decrease in the GMT of neutralizing capacities of circulating antibodies against SARS-CoV2 twenty weeks after the second dose (**Figure 2**). Due to this decrease in neutralizing capacities, a booster dose of CoronaVac® was evaluated in a clinical study in China, showing promising results in enhanced humoral immune responses[12,26]. The evaluation of the immune response reported here shows that after the booster dose, the neutralizing titers and seroconversion rates increase in the whole group, to a higher extent than two weeks after the second dose, where the peak in neutralization was previously observed, which is in line with the observed by *Clemens et al.*,[15] . Also, we observed a steady activation of the $CD4^+$ T cells and secretion of IFN-γ during the time-points evaluated (**Figure 3**).

Since the neutralizing antibody titers correlated with protection against SARS-CoV-2 infection [10], these results likely imply a better outcome and protection against COVID-19, as reported in previous studies performed in Israel that showed a decrease in the transmission and the disease severity disease by this virus twelve or more days after booster inoculation. In Chile, the effectiveness and prevention in hospitalization increased when assessed fourteen days after the booster dose of CoronaVac® [19,27]. Another study, performed with a booster dose of CoronaVac®, showed that an additional dose result in good neutralization capacity against parental SARS-CoV-2 and against Delta variant four weeks after the booster dose, generating a long-lasting humoral response, which was due to an enhancement of the memory immune response generated by B cells [26].

Adults ≥60 years old produced lower levels of antibodies with neutralizing capacities than the whole group during this study, which was also described previously (**Figure 2C and F**) [5]. This result is in line with previous data reported for a population vaccinated in Chile [6], among hospital workers who received two doses of CoronaVac® [28], and in a study with the mRNA-1273 vaccine [29]. In this sense, our results are equivalent to those described in a phase I/II of the clinical trial performed with CoronaVac® in China, showing that the neutralizing antibody titers in this group decreased at five months after the second dose and that a booster dose is required 6-8 months after the first vaccination to rapidly increase and maintain the neutralizing antibody titers [30].

In the case of the T cell response (**Figure 3**), other studies have shown that Pfizer BNT162b2 and mRNA-1273 induce durable $CD4^+$ T cell activation and cytokine production up to six months following vaccination, but it remains to be

elucidated whether expression of AIM by $CD4^+$ T cells and cytokine production further increase following a booster dose with these vaccines [31,32]. Here, we observed that the activation of $CD4^+$ T cells and IFN-γ production stays increased up to twenty weeks after the second dose, and after the booster dose both parameters increased in the 18-59 years old group and was maintained at the levels observed twenty weeks after the second dose in adults ≥60 years old. In contrast to BNT162b2 and mRNA-1273 vaccines, CoronaVac® delivers not only the Spike protein upon immunization but also other viral antigens, which may explain why vaccinated individuals still display AIM^+ $CD4^+$ T cells five months after the second dose, regardless of a third dose.

When the neutralization capacity analyzed by pVNT of the VOC Delta and Omicron was evaluated four weeks after the booster dose, we observed differences in the neutralization capacity as compared to the D614G variant (lineage B.1), which does not exhibit mutations in the RBD of the S1 protein (**Figure 4 and table 3**). We previously reported that CoronaVac® is able to induce neutralization against the Delta variant at 4 weeks after the second dose, although to a lesser extent compared to the WT strain [14]. Another study recently reported a significant increase in the neutralizing capacity after a booster dose with CoronaVac® for the Delta variant, as compared to the levels observed in volunteers vaccinated with two doses [26,33]. Although we did not observe similar levels of enhanced neutralization against the variants Delta after the booster dose using pVNT (**Figure 4A**), the seropositivity against Delta variant is almost 100% (**Figure 4B**)[33]. Here, we also show that a booster dose induces neutralization against the

393 variant Omicron, which has rapidly spread worldwide and is the predominant
394 circulating variant to date [34]. The high number of mutations described in the RBD of
395 this variant has been associated with increased evasion of neutralizing responses
396 in either unvaccinated or vaccinated subjects [34,35]. Although the neutralization
397 observed in subjects vaccinated with a booster dose of CoronaVac® is significantly
398 lower to the observed against the D614G variant, we observed a seropositivity of
399 76.7% following the booster, suggesting some degree of protection in most of the
400 vaccinees. In this sense, it has been reported that a heterologous schedule of
401 vaccination may induce a higher neutralization ability and a better neutralization
402 against variants of concern as Delta [36] and Omicron[16]. In line with this, a
403 heterologous vaccination with CoronaVac® and a booster dose of Pfizer BNT162b2
404 showed a good neutralization titer against VOC Delta and Omicron, with respect to
405 the ancestral virus [16]. Similarly, a comparison between heterologous and
406 homologous booster schedules after the vaccination with CoronaVac®, shows an
407 increase in neutralization against the VOC Delta and Omicron[15]. There are
408 discrepancies between the results in neutralization titers, which can be attributed to
409 the neutralization assays performed and/or the study population; however,
410 important booster responses are observed in these studies, and seropositivity
411 reached after the booster dose of CoronaVac® against VOC are also similar [16].

412 In the case of the cellular response, this is the first report to characterize
413 $CD4^+$ T cell responses following a booster dose of CoronaVac® against the
414 Omicron variant of SARS-CoV2. Previous studies using the same MP from VOC
415 evaluated here have shown that $CD4^+$ T cells respond to VOC in a similar extent
416 as compared to the ancestral strain in individuals vaccinated with CoronaVac® [14,37]

and mRNA vaccines, which has been explained by the high conservation of T cell epitopes. In this sense, the booster dose of CoronaVac® induces the expression of $CD4^+$ T cell activation markers and secretion of IFN-γ and IL-2 against the VOC Delta and Omicron, which is comparable to the response generated against the WT strain (**Figure 5**). In line with this, a recent study has shown that T cell responses against the ancestral strain are cross-reactive against the Omicron variant in convalescent individuals and volunteers vaccinated with Pfizer BNT162b2 [38], supporting the idea that the induction of T cell responses against the ancestral strain may be protective against the Omicron variant.

Our report shows that the booster dose of CoronaVac® in a 0-28 days schedule induces antibodies with neutralizing capacities, which are higher than the levels observed at 2- and 4-weeks after the second dose, generating an increased humoral response even in adults ≥60 years old. Besides this, our results suggest that a third dose of CoronaVac® supports $CD4^+$ T cell activation, which may confer either protection or enhanced immune responses against the virus and prevent severe disease following exposure to SARS-CoV-2 exposure. Importantly, the humoral and cellular immune response promoted by a booster dose of CoronaVac shows activity against Delta and Omicron variants and probably results in better effectiveness of this vaccine during predominance of these VOC.

Limitations

This study presents several limitations, such as the reduced sample size for the assays and the absence of data for neutralization against Omicron variant with

a conventional viral neutralization test. The assessment of total antibody response against Spike proteins and other SARS-CoV-2 proteins would also add additional information about the humoral immune response against SARS-CoV-2 after the booster dose. Due to the limit of quantification of the technique, samples with undetermined concentration at the lowest dilution tested (1:4) were assigned the lower limit of quantification (16.4 IU) and other neutralization assays.

Funding

The CoronaVac03CL Study was funded by The Ministry of Health, Government of Chile, the Confederation of Production and Commerce (CPC), Chile and SINOVAC Biotech. NIH NIAID, under Contract 75N93021C00016, supports AS and Contract 75N9301900065 supports AS, AG and DW. The National Agency for Research and Development (ANID) through the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) grants Nº 1190156 and Nº 1211547 supports RSR and FVE, respectively. The Millennium Institute on Immunology and Immunotherapy, ANID - Millennium Science Initiative Program ICN09_016 (former P09/016-F) supports RSR, VFE, SMB, KA, PAG and AMK; The Innovation Fund for Competitiveness FIC-R 2017 (BIP Code: 30488811-0) supports SMB, PAG and AMK.

Competing interests

ZG and MW are SINOVAC Biotech employees and contributed to the conceptualization of the study (clinical protocol and eCRF design) and did not participate in the analysis or interpretation of the data presented in the manuscript.

A.S. is a consultant for Gritstone, Flow Pharma, Arcturus, Immunoscape, CellCarta, OxfordImmunotech and Avalia. La Jolla Institute for Immunology (LJI) has filed for patent protection for various aspects of T cell epitope and vaccine design work. All other authors declare no conflict of interest.

Acknowledgments

We would like to thank the support of the Ministry of Health, Government of Chile; Ministry of Science, Technology, Knowledge, and Innovation, Government of Chile; The Ministry of Foreign Affairs, Government of Chile, and the Chilean Public Health Institute (ISP). We also would like to thank Rami Scharf, Jessica White, Jorge Flores and Miren Iturriza-Gomara from PATH for their support on experimental design and discussion. Alex Cabrera and Sergio Bustos from the Flow Cytometry Facility at Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile for support with flow cytometry. We also thank the Vice Presidency of Research (VRI), the Direction of Technology Transfer and Development (DTD), the Legal Affairs Department (DAJ) of the Pontificia Universidad Católica de Chile. We are also grateful to the Administrative Directions of the School of Biological Sciences and the School of Medicine of the Pontificia Universidad Católica de Chile for their administrative support. We would also like to thank the members of the independent data safety monitoring committee (members in the SA) for their oversight, and the subjects enrolled in the study for their participation and commitment to this trial. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases,

National Institutes of Health, Department of Health and Human Services, under Contract No. Contract No. 75N9301900065 to A.S, A.G. and D.W.

Note: Members of the CoronaVac03CL Study Group are listed in the Supplementary Appendix (SA).

References

1. Mallapaty S. WHO approval of Chinese CoronaVac COVID vaccine will be crucial to curbing pandemic [Internet]. Nature. 2021 [cited 2021 Oct 6];594(7862):161–2. Available from: https://pubmed.ncbi.nlm.nih.gov/34089030/
2. Q G, L B, H M, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science [Internet] 2020 [cited 2021 Oct 6];369(6499):77–81. Available from: https://pubmed.ncbi.nlm.nih.gov/32376603/
3. Z W, Y H, M X, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis [Internet] 2021 [cited 2021 Oct 6];21(6):803–12. Available from: https://pubmed.ncbi.nlm.nih.gov/33548194/
4. Y Z, G Z, H P, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis [Internet] 2021 [cited 2021 Oct 6];21(2):181–92. Available from: https://pubmed.ncbi.nlm.nih.gov/33217362/
5. SM B, K A, PA G, et al. Safety and Immunogenicity of an Inactivated SARS-CoV-2 Vaccine in a Subgroup of Healthy Adults in Chile. Clin Infect Dis [Internet] 2021 [cited 2021 Oct 9];Available from: https://pubmed.ncbi.nlm.nih.gov/34537835/
6. Sauré D, O'Ryan M, Torres JP, Zuniga M, Santelices E, Basso LJ. Dynamic IgG seropositivity after rollout of CoronaVac and BNT162b2 COVID-19

vaccines in Chile: a sentinel surveillance study. Lancet Infect Dis [Internet] 2021 [cited 2021 Oct 11];0(0). Available from: http://www.thelancet.com/article/S1473309921004795/fulltext

7. Duarte LF, Gálvez NMS, Iturriaga C, et al. Immune Profile and Clinical Outcome of Breakthrough Cases After Vaccination With an Inactivated SARS-CoV-2 Vaccine. Front Immunol [Internet] 2021 [cited 2021 Oct 11];0:3884. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.742914/full

8. Jara A, Undurraga EA, González C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. https://doi.org/101056/NEJMoa2107715 [Internet] 2021 [cited 2021 Oct 7];385(10):875–84. Available from: https://www.nejm.org/doi/full/10.1056/NEJMoa2107715

9. Chile ha administrado más de 41 millones 980 mil dosis de vacuna contra SARS-CoV-2 - Ministerio de Salud - Gobierno de Chile [Internet]. [cited 2021 Dec 10];Available from: https://www.minsal.cl/chile-ha-administrado-mas-de-41-millones-980-mil-dosis-de-vacuna-contra-sars-cov-2/

10. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med 2021 277 [Internet] 2021 [cited 2021 Oct 6];27(7):1205–11. Available from: https://www.nature.com/articles/s41591-021-01377-8

11. Protection duration after vaccination or infection – Swiss National COVID-19 Science Task Force [Internet]. [cited 2021 Oct 6];Available from: https://sciencetaskforce.ch/en/policy-brief/protection-duration-after-vaccination-or-infection/

12. Pan H, Wu Q, Zeng G, et al. Immunogenicity and safety of a third dose, and immune persistence of CoronaVac vaccine in healthy adults aged 18-59 years: interim results from a double-blind, randomized, placebo-controlled phase 2 clinical trial. medRxiv [Internet] 2021 [cited 2021 Oct 6];2021.07.23.21261026. Available from: https://www.medrxiv.org/content/10.1101/2021.07.23.21261026v1

13. Cao Y, Yisimayi A, Bai Y, et al. Humoral immune response to circulating SARS-CoV-2 variants elicited by inactivated and RBD-subunit vaccines. Cell Res 2021 317 [Internet] 2021 [cited 2021 Oct 8];31(7):732–41. Available from: https://www.nature.com/articles/s41422-021-00514-9

14. Melo-González F, Soto JA, González LA, et al. Recognition of Variants of Concern by Antibodies and T Cells Induced by a SARS-CoV-2 Inactivated Vaccine. Front Immunol [Internet] 2021;12. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.747830/full

15. Clemens SAC, Weckx L, Clemens R, et al. Heterologous versus homologous COVID-19 booster vaccination in previous recipients of two doses of CoronaVac COVID-19 vaccine in Brazil (RHH-001): a phase 4, non-inferiority, single blind, randomised study. Lancet [Internet] 2022 [cited 2022 Jan 24];0(0). Available from: http://www.thelancet.com/article/S0140673622000940/fulltext

16. Pérez-Then E, Lucas C, Monteiro VS, et al. Neutralizing antibodies against the SARS-CoV-2 Delta and Omicron variants following heterologous CoronaVac plus BNT162b2 booster vaccination. Nat Med 2022 [Internet] 2022 [cited 2022 Jan 24];1–1. Available from:

https://www.nature.com/articles/s41591-022-01705-6

17. Acevedo M, Alonso-Palomares L, Pantano S, Pasteur I, Navarrete M, Soto-Rifo R. Neutralization of the emerging SARS-CoV-2 variant Lambda by antibodies elicited by inactivated virus and mRNA vaccines. 2021 [cited 2022 Jan 31];Available from: https://www.researchsquare.com

18. Depto Inmunizaciones D, De Prevención D, Control, Y Enfermedades S, Pública M de salud. DOSIS DE REFUERZO EN LA CAMPAÑA DE VACUNACIÓN CONTRA SARS-COV-2 EN CHILE. 2021 [cited 2021 Oct 6];Available from: https://www.minsal.cl/wp-

19. Immunization Campaign against SARS-CoV-2 Early estimates of the effectiveness of booster shots in Chile [Internet]. 2021 [cited 2021 Dec 10];Available from: https://www.minsal.cl/wp-content/uploads/2021/10/2021-10-07-EFECTIVIDAD-DOSIS-DE-REFUERZO_ENG.pdf

20. World medical association. World Medical Association declaration of Helsinki: Ethical principles for medical research involving human subjects [Internet]. JAMA - J. Am. Med. Assoc. 2013 [cited 2021 Oct 6];310(20):2191–4. Available from: https://pubmed.ncbi.nlm.nih.gov/24141714/

21. WHO/BS.2020.2403 Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody [Internet]. [cited 2021 Oct 8];Available from: https://www.who.int/publications/m/item/WHO-BS-2020.2403

22. Acevedo ML, Alonso-Palomares L, Bustamante A, et al. Infectivity and immune escape of the new SARS-CoV-2 variant of interest Lambda. medRxiv [Internet] 2021 [cited 2022 Jan 24];2021.06.28.21259673. Available

588 from: https://www.medrxiv.org/content/10.1101/2021.06.28.21259673v1
589 23. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T Cell Responses to
590 SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and
591 Unexposed Individuals. Cell 2020;181(7):1489-1501.e15.
592 24. Lumley SF, O'Donnell D, Stoesser NE, et al. Antibody Status and Incidence
593 of SARS-CoV-2 Infection in Health Care Workers.
594 https://doi.org/101056/NEJMoa2034545 [Internet] 2020 [cited 2021 Oct
595 8];384(6):533–40. Available from:
596 https://www.nejm.org/doi/10.1056/NEJMoa2034545
597 25. Sabino EC, Buss LF, Carvalho MPS, et al. Resurgence of COVID-19 in
598 Manaus, Brazil, despite high seroprevalence. Lancet 2021;397(10273):452–
599 5.
600 26. Wang K, Cao Y, Zhou Y, et al. A third dose of inactivated vaccine augments
601 the potency, breadth, and duration of anamnestic responses against SARS-
602 CoV-2. medRxiv [Internet] 2021 [cited 2021 Oct 8];2021.09.02.21261735.
603 Available from:
604 https://www.medrxiv.org/content/10.1101/2021.09.02.21261735v1
605 27. Bar-On YM, Goldberg Y, Mandel M, et al. BNT162b2 vaccine booster dose
606 protection: A nationwide study from Israel. medRxiv [Internet] 2021 [cited
607 2021 Oct 8];2021.08.27.21262679. Available from:
608 https://www.medrxiv.org/content/10.1101/2021.08.27.21262679v1
609 28. Seyahi E, Bakhdiyarli G, Oztas M, et al. Antibody response to inactivated
610 COVID-19 vaccine (CoronaVac) in immune-mediated diseases: a controlled
611 study among hospital workers and elderly. Rheumatol Int 2021 418 [Internet]

612 2021 [cited 2021 Oct 8];41(8):1429–40. Available from:
613 https://link.springer.com/article/10.1007/s00296-021-04910-7
614 29. Widge AT, Rouphael NG, Jackson LA, et al. Durability of Responses after
615 SARS-CoV-2 mRNA-1273 Vaccination.
616 https://doi.org/101056/NEJMc2032195 [Internet] 2020 [cited 2021 Oct
617 8];384(1):80–2. Available from:
618 https://www.nejm.org/doi/full/10.1056/NEJMc2032195
619 30. Li M, Yang J, Wang L, et al. A booster dose is immunogenic and will be
620 needed for older adults who have completed two doses vaccination with
621 CoronaVac: a randomised, double-blind, placebo-controlled, phase 1/2
622 clinical trial. medRxiv [Internet] 2021 [cited 2021 Oct
623 8];2021.08.03.21261544. Available from:
624 https://www.medrxiv.org/content/10.1101/2021.08.03.21261544v1
625 31. Mateus J, Dan JM, Zhang Z, et al. Low-dose mRNA-1273 COVID-19 vaccine
626 generates durable memory enhanced by cross-reactive T cells. Science (80-
627) [Internet] 2021 [cited 2021 Nov 11];374(6566). Available from:
628 https://www.science.org/doi/abs/10.1126/science.abj9853
629 32. Guerrera G, Picozza M, D'Orso S, et al. BNT162b2 vaccination induces
630 durable SARS-CoV-2 specific T cells with a stem cell memory phenotype.
631 Sci Immunol [Internet] 2021 [cited 2021 Nov 11];Available from:
632 https://www.science.org/doi/abs/10.1126/sciimmunol.abl5344
633 33. Cao Y, Hao X, Wang X, et al. Humoral immunogenicity and reactogenicity of
634 CoronaVac or ZF2001 booster after two doses of inactivated vaccine. Cell
635 Res 2021 [Internet] 2021 [cited 2021 Dec 10];1–3. Available from:

https://www.nature.com/articles/s41422-021-00596-5

34. Viana R, Moyo S, Amoako DG, et al. Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. Nat 2022 [Internet] 2022 [cited 2022 Jan 31];1–10. Available from: https://www.nature.com/articles/s41586-022-04411-y

35. Hoffmann M, Krüger N, Schulz S, et al. The Omicron variant is highly resistant against antibody-mediated neutralization: Implications for control of the COVID-19 pandemic. Cell [Internet] 2021 [cited 2022 Jan 25];Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867421014951

36. Ai J, Zhang H, Zhang Q, et al. Recombinant protein subunit vaccine booster following two-dose inactivated vaccines dramatically enhanced anti-RBD responses and neutralizing titers against SARS-CoV-2 and Variants of Concern. Cell Res 2021 [Internet] 2021 [cited 2021 Dec 10];1–4. Available from: https://www.nature.com/articles/s41422-021-00590-x

37. A T, J S, N M, et al. Impact of SARS-CoV-2 variants on the total CD4 + and CD8 + T cell reactivity in infected or vaccinated individuals. Cell reports Med [Internet] 2021 [cited 2021 Oct 25];2(7). Available from: https://pubmed.ncbi.nlm.nih.gov/34230917/

38. Gao Y, Cai C, Grifoni A, et al. Ancestral SARS-CoV-2-specific T cells cross-recognize the Omicron variant. Nat Med 2022 [Internet] 2022 [cited 2022 Jan 25];1–9. Available from: https://www.nature.com/articles/s41591-022-01700-x

660 **Figures legends**

661

662 **Figure 1: Study profile, enrolled volunteers, and cohort included in the study**
663 **by November 11th, 2021. A.** From the one hundred and eighty-six vaccinated
664 individuals that received the booster dose, seventy-seven volunteers that received
665 two doses of CoronaVac® in a 28 days interval (0-28 days schedule of
666 vaccination), were selected from the center assigned for the immunogenicity study.
667 Seventy-seven volunteers were tested for neutralizing antibodies by sVNT, sixty-
668 two were selected to analyze neutralizing antibodies by cVNT and forty were
669 selected to analyze cellular immunity. Analyses for immunity against SARS-CoV-2
670 variants were performed in 30 volunteers for sVNT, pVNT and T cells assays. **B.**
671 Timeline of 0–28 days schedule of vaccination and booster dose immunization.
672 Text in red denotes timepoints at which blood draws occurred.

673

674 **Figure 2: Quantification of circulating antibodies inhibiting the interaction**
675 **between the S1-RBD and hACE2 and in live SARS-CoV-2 in volunteers that**
676 **received the booster dose of CoronaVac®. A-C.** Inhibiting antibodies were
677 detected in serum of volunteers immunized with CoronaVac® using a surrogate
678 Viral Neutralization Test (sVNT), which quantifies the interaction between S1-RBD
679 and hACE2 on ELISA plates. Results were obtained from a total of seventy-seven
680 volunteers (**A**), thirty-six of them were adults between 18-59 years old (**B**), and
681 forty-one of them were ≥ 60 years old (**C**). Data is represented as WHO arbitrary
682 units/mL, the numbers above each set of individual data points show the
683 Geometric Mean Units (GMU), the error bars indicate the 95% CI, and the number

684 at the right represents the fold increase of the GMU four weeks after the third dose
685 as compared with the respective times after administration of the second dose. **D-**
686 **F**. Neutralizing antibodies were detected in serum of volunteers that received a
687 booster dose of CoronaVac® twenty weeks after the second dose, using a
688 conventional Viral Neutralization Test (cVNT), which quantifies the reduction of
689 cytopathic effect (CPE) in Vero E6 cells infected with SARS-CoV-2. Results were
690 obtained from 62 volunteers (**D**), 30 of them were adults between 18-59 years old
691 (**E**), and 32 of them were ≥ 60 years old (**F**). Data are expressed as the reciprocal
692 of the highest serum dilution preventing 100% cytopathic effect, the numbers
693 above each set of individual data points show the Geometric Mean Titer (GMT),
694 the error bars indicate the 95% CI, and the number at the right represents the fold
695 increase of the GMU the third dose + 4 weeks as compared with the respective
696 times after administration of the second dose. CI were not adjusted for multiplicity
697 and should not be used for inference. A repeated measures One-Way ANOVA test
698 assessed statistical differences to compare all times against 3rd dose + four weeks.
699 ****p<0.0001.
700
701 **Figure 3: Changes in activation-induced markers (AIMs) expression in CD4$^+$ T**
702 **cells and in the number of IFN-γ-secreting cells specific for SARS-CoV-2 after**
703 **a booster dose of CoronaVac®. A-C.** AIM$^+$ CD4$^+$ T cells were quantified in
704 peripheral blood mononuclear cells of volunteers that received a booster dose of
705 CoronaVac® twenty weeks after the second dose by flow cytometry, upon
706 stimulation with mega-pools of peptides derived from SARS-CoV-2 proteins. The

percentage of activated AIM$^+$ CD4$^+$ T cells (OX40$^+$, CD137$^+$) were determined upon stimulation for 24h with MP-S+R in samples obtained at pre-immune, two weeks after the second dose, four weeks after the second dose, twenty weeks the second dose, and four weeks after the booster dose. Data from flow cytometry was normalized against DMSO and analyzed separately by a Friedman test against the booster dose. Results were obtained from a total of forty volunteers (**A**), twenty-one were of them were adults between 18-59 years old (**B**), and nineteen of them were ≥ 60 years old (**C**). Changes in the secretion of IFN-γ were quantified as the number of Spot Forming Cells (SFCs) in peripheral blood mononuclear cells of volunteers that received a booster dose of CoronaVac® 20 weeks after the second dose. **D-F**. Data was obtained upon stimulation with MP-S+R for 48h in samples obtained at pre-immune, two weeks after the second dose, four weeks after the second dose, twenty weeks the second dose, and four weeks after the booster dose. Results were obtained from a total of 40 volunteers (**D**), 21 were of them were adults between 18-59 years old (**E**), and 19 of them were ≥ 60 years old (**F**). Data from ELISPOT were analyzed separately by Friedman test against the booster dose *p<0.05; **p<0.005; ***p< < 0.001; ****p<0.0001.

Figure 4: Quantification of circulating neutralizing antibodies against SARS-CoV-2 variants in volunteers that received the booster dose of CoronaVac®. **A.** Neutralizing antibodies were detected in the serum of thirty volunteers, four weeks after the booster dose of CoronaVac®, using a pseudotyped virus neutralization test (pVNT). Data are expressed as the reciprocal of the highest

730 dilution preventing 80% of the infection (ID80). Numbers above the bars show the
731 Mean, and the error bars indicate the 95% CI. The number at the right represents
732 the fold decrease of the GMT four weeks after the booster dose as compared with
733 the response of D614G **B**. Seropositivity rate of neutralizing antibodies is shown for
734 each time point analyzed. Numbers above the bars show the percentage of
735 seropositivity rate in the respective graphs. Numbers above the bars show the
736 percentage of seropositivity rate in the respective graphs. A repeated measures
737 One-Way ANOVA test assessed statistical differences of the GMT to compare
738 each variant against D614G. *p<0.05; ***p < 0.001; ****p<0.0001
739
740
741 **Figure 5: A booster dose of CoronaVac® induce changes in the number of**
742 **IFN-γ-secreting cells and in activation-induced markers (AIMs) expression in**
743 **$CD4^+$ T cells specific for the Spike protein of SARS-CoV-2 variants. A.**
744 Changes in the secretion of IFN-γ, determined as the number of Spot Forming
745 Cells (SFCs) were determined. Data was obtained upon stimulation of PBMC with
746 MP-S of variant of concern of SARS-CoV-2 for 48h in samples obtained four weeks
747 after the booster dose. Data shown represent mean + 95%CI. Data from thirty
748 volunteers were analyzed at four weeks after the booster dose to compare among
749 the MP-S of the variant of concern. Data from ELISPOT were analyzed separately
750 by Friedman test against the WT MP-S. No significant differences were obtained.
751 **B**. AIM^+ $CD4^+$ T cells were quantified in peripheral blood mononuclear cells of thirty
752 volunteers four weeks after that received a booster dose of CoronaVac® by flow

753 cytometry, upon stimulation with mega-pools of peptides derived from proteins of
754 variant of concern of SARS-CoV-2. The percentage of activated AIM^+ $CD4^+$ T cells
755 ($OX40^+$, $CD137^+$) were determined upon stimulation for 24h with MP-S+R in
756 samples obtained four weeks after the booster dose. Data shown represent mean
757 + 95%CI. Data from flow cytometry was normalized against DMSO. No significant
758 differences were obtained between WT and the variant MP stimulation.
759

Table

Table 1: Demographic and clinical data of seventy-seven volunteers analyzed.

	Age group	AHT N (%)	AR N (%)	MD N (%)	Obesity N (%)	Insulin resistance N (%)	COPD N (%)	HT N (%)
Female sex N (%)	41 (53.2)	11 (14.3)	8 (10.4)	1 (1.3)	6 (7.8)	6 (7.8)	3 (3.9)	7 (9.1)
18-59 years old	18 (23.4)	3 (3.9)	6 (7.8)	0	2 (2.6)	3 (3.9)	0	2 (2.6)
≥60 years old	23 (30.0)	8 (10.4)	2 (2.6)	1 (1.3)	4 (5.2)	3 (3.9)	3 (3.9)	5 (6.5)
Male sex N (%)	36 (46.8)	11 (14.3)	8 (10.4)	3 (3.9)	11 (14.3)	1 (1.3)	0	1 (1.3)
18-59 years old	17 (22.0)	4 (5.2)	4 (5.2)	2 (2.6)	4 (5.2)	0	0	0
≥60 years old	19 (24.6)	7 (9.1)	4 (5.2)	1 (1.3)	7 (9.1)	1 (1.3)	0	1 (1.3)

Arterial hypertension: AHT; Chronic obstructive pulmonary disease: COPD; Mellitus diabetes: MD; Hypothyroidism: HT; Allergic rhinitis: AR

768 **Table 2: Seropositivity rates, Geometric Mean Titer (GMT), and Geometric**
769 **Mean Units (GMU) of circulating neutralizing antibodies against SARS-CoV-2**
770 **RBD.**

Methodology	Age group	Indicators	2nd dose + 2 weeks	2nd dose + 4 weeks	2nd dose + 20 weeks	3rd dose + 4 weeks
sVNT	Total Vaccine	Seropositivity n/N	72/77	73/77	38/77	75/77
		(%)	93.5	94.8	49.4	97.4
		GMU	168.0	124.8	39.0	499.4
		95% CI	126.8-222.5	96.3-161.7	32.4-47.0	370.6-673.0
		GMT	25.8	16.6	3.5	53.0
		95% CI	19.5-34.2	13.1-21.0	3.0-4.1	40.8-68.8
	18-59	Seropositivity n/N	35/36	36/36	24/36	36/36
		(%)	97.2	97.2	66.7	100
		GMU	220.2	155.0	48.9	918.8
		95% CI	150.7-321.7	108.0-222.6	37.6-63.5	623.4-1354
		GMT	33.3	19.1	4.3	82.8
		95% CI	23.4-47.3	14.0-26.1	3.4-5.4	59.7-114.8
	≥60	Seropositivity n/N	38/41	39/42	15/42	40/42
		(%)	90.5	92.9	35.7	95.2
		GMU	134.1	104.1	32.4	300.5
		95% CI	89.2-201.6	71.8-151.0	25.1-41.8	203.5-443.6
		GMT	20.8	14.7	2.4	36.5
		95% CI	13.6-31.9	10.3-21.0	2.4-3.5	25.3-52.7
cVNT	Total Vaccine	Seropositivity n/N	49/62	51/62	44/62	62/72
		(%)	79.0	82.3	71.0	100
		GMT	12.8	13.5	8.3	89.5
		95% CI	8.8-18.5	9.6-19.2	5.6-12.2	64.0-125.2
	18-59	Seropositivity n/N	25/30	27/30	23/30	30/30
		(%)	83.3	90.0	76.7	100

		GMT	17.5	18.8	14.2	176.9
		95% CI	9.8-31.3	11.2-31.7	7.1-28.4	111.7-280.1
	≥60	Seropositivity n/N	24/32	24/32	21/32	32/32
		(%)	75.0	75.0	65.6	100
		GMT	9.5	9.9	5.0	47.3
		95% CI	5.8-15.4	6.2-15.8	3.5-7.0	32.1-69.5

sVNT: Surrogate Virus Neutralization; cVNT: Conventional Virus Neutralization; GMT: Geometric mean titer; GMU: Geometric mean units.

773 **Table 3: Seropositivity rates, Geometric Mean Titer (GMT) of circulating**
774 **neutralizing antibodies against SARS-CoV-2 RBD of D614G and variants of**
775 **concern (Delta and Omicron).**
776

	Variant	D614G	Delta (B.1.617.2)	Omicron (B.1.1.529)
pVNT	Indicators	3rd dose + 4 weeks	3rd dose + 4 weeks	3rd dose + 4 weeks
	Seropositivity n/N	30/30	28/30	23/30
	(%)	100	93.3	76.6
	GMT	241.8	159.2	50.7
	95% CI	155.7-375.6	99.1-256.0	30.4-84.8

777

778 *GMT: Geometric mean titer.*

779

780

Figure 1

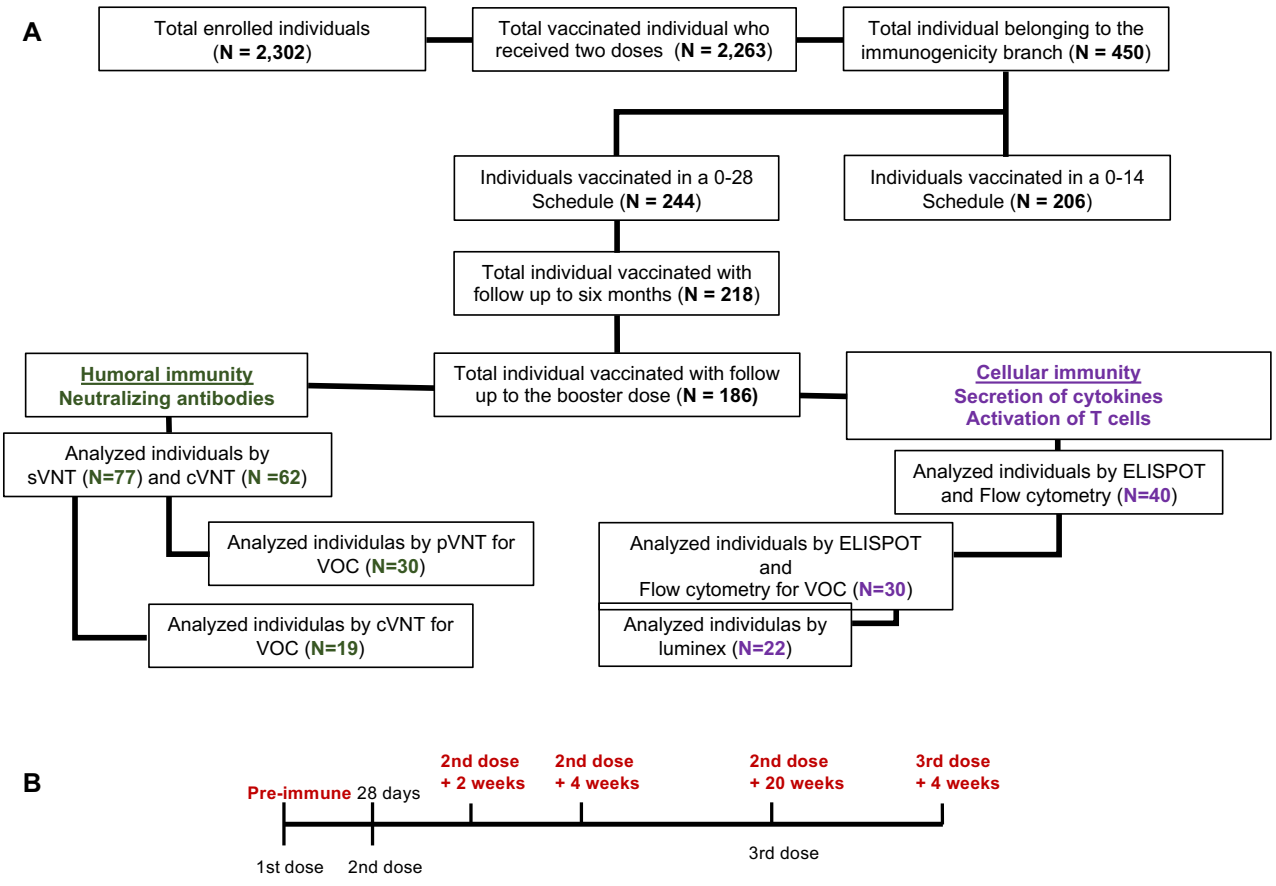

A
Total enrolled individuals (N = 2,302)
Total vaccinated individual who received two doses (N = 2,263)
Total individual belonging to the immunogenicity branch (N = 450)
Individuals vaccinated in a 0-28 Schedule (N = 244)
Individuals vaccinated in a 0-14 Schedule (N = 206)
Total individual vaccinated with follow up to six months (N = 218)
Humoral immunity
Neutralizing antibodies
Total individual vaccinated with follow up to the booster dose (N = 186)
Cellular immunity
Secretion of cytokines
Activation of T cells
Analyzed individuals by sVNT (N=77) and cVNT (N =62)
Analyzed individuals by ELISPOT and Flow cytometry (N=40)
Analyzed individulas by pVNT for VOC (N=30)
Analyzed individuals by ELISPOT and Flow cytometry for VOC (N=30)
Analyzed individulas by cVNT for VOC (N=19)
Analyzed individulas by luminex (N=22)
B
Pre-immune
28 days
2nd dose + 2 weeks
2nd dose + 4 weeks
2nd dose + 20 weeks
3rd dose + 4 weeks
1st dose
2nd dose
3rd dose

Figure 2

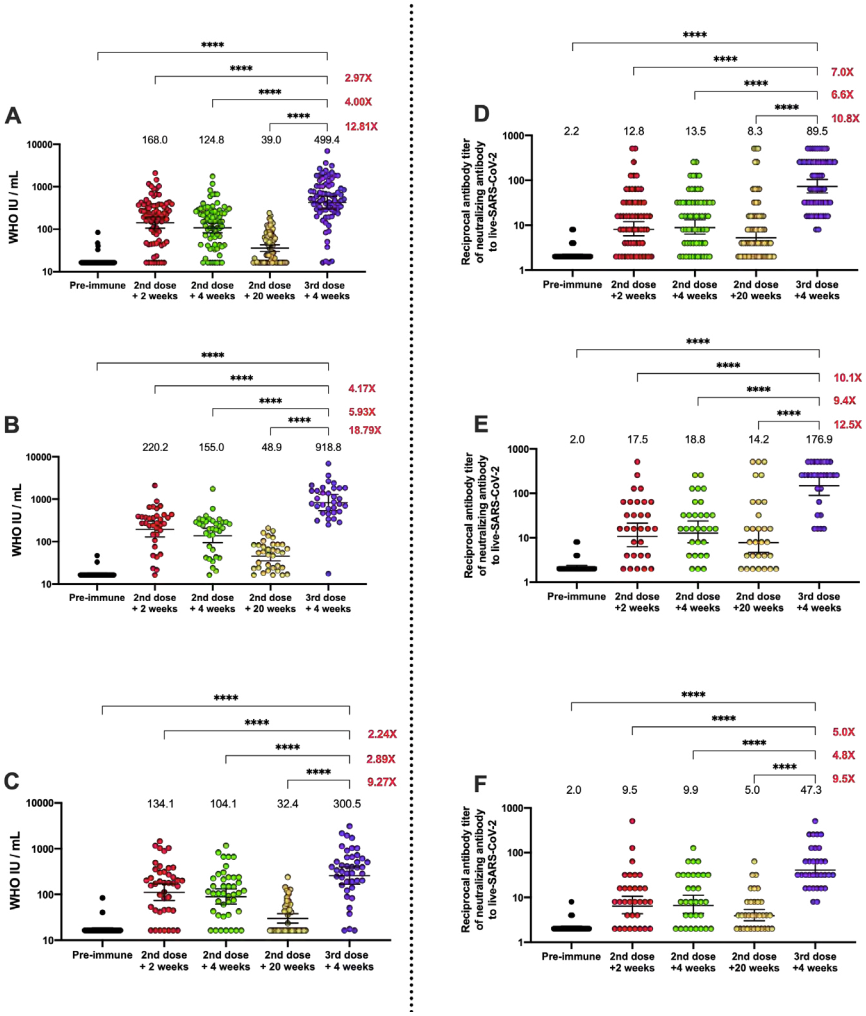

A
WHO IU / mL
168.0
124.8
39.0
499.4
2.97X
4.00X
12.81X
B
220.2
155.0
48.9
918.8
4.17X
5.93X
18.79X
C
134.1
104.1
32.4
300.5
2.24X
2.89X
9.27X
D
Reciprocal antibody titer of neutralizing antibody to live-SARS-CoV-2
2.2
12.8
13.5
8.3
89.5
7.0X
6.6X
10.8X
E
2.0
17.5
18.8
14.2
176.9
10.1X
9.4X
12.5X
F
2.0
9.5
9.9
5.0
47.3
5.0X
4.8X
9.5X
****
Pre-immune
2nd dose + 2 weeks
2nd dose + 4 weeks
2nd dose + 20 weeks
3rd dose + 4 weeks
2nd dose +2 weeks
2nd dose +4 weeks
2nd dose +20 weeks
3rd dose +4 weeks

Figure 3

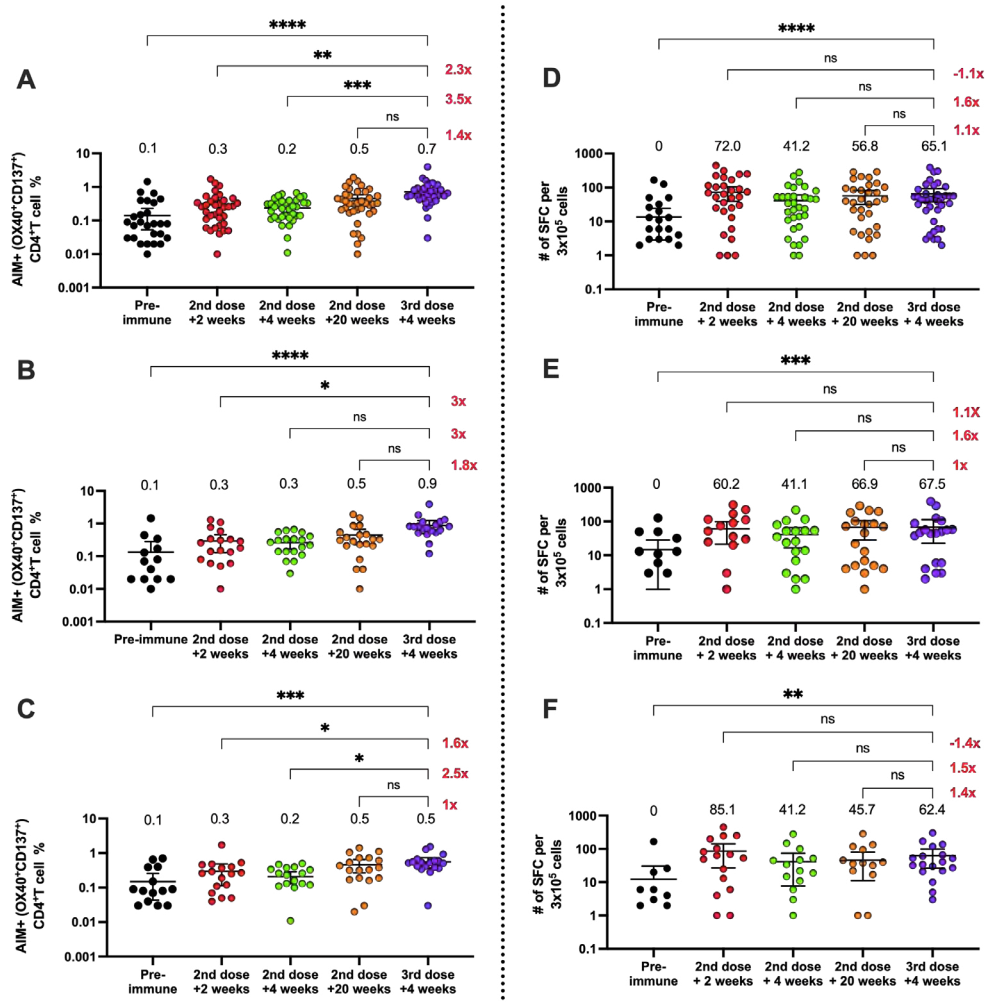

A
B
C
D
E
F
AIM+ (OX40+CD137+) CD4+T cell %
# of SFC per 3x10^5 cells
Pre-immune
2nd dose +2 weeks
2nd dose +4 weeks
2nd dose +20 weeks
3rd dose +4 weeks
2nd dose + 2 weeks
2nd dose + 4 weeks
2nd dose + 20 weeks
3rd dose + 4 weeks
ns
2.3x
3.5x
1.4x
3x
1.8x
1.6x
2.5x
1x
-1.1x
1.6x
1.1x
1.1X
-1.4x
1.5x

Figure 4

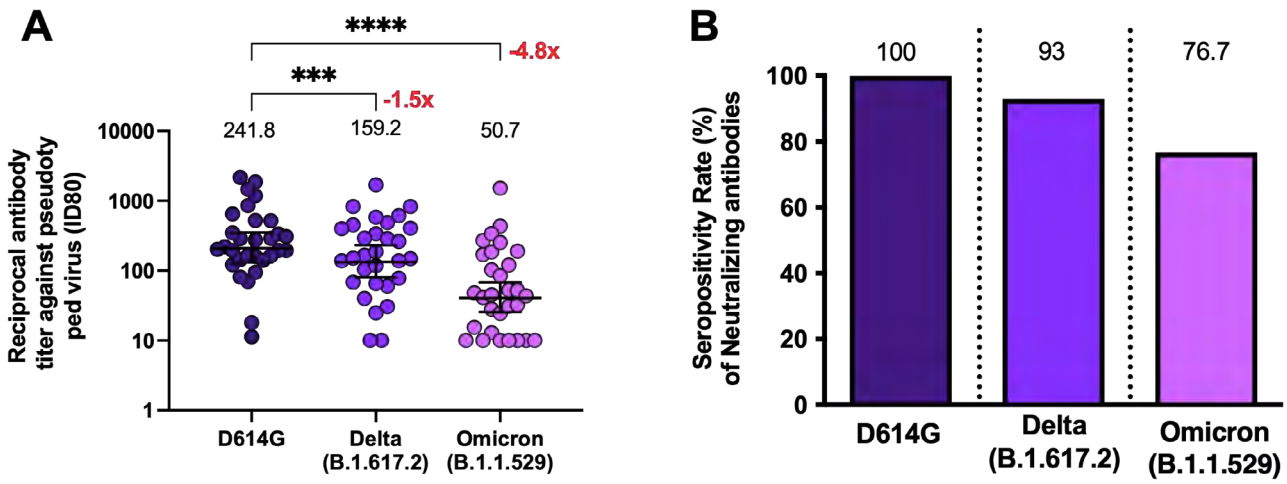
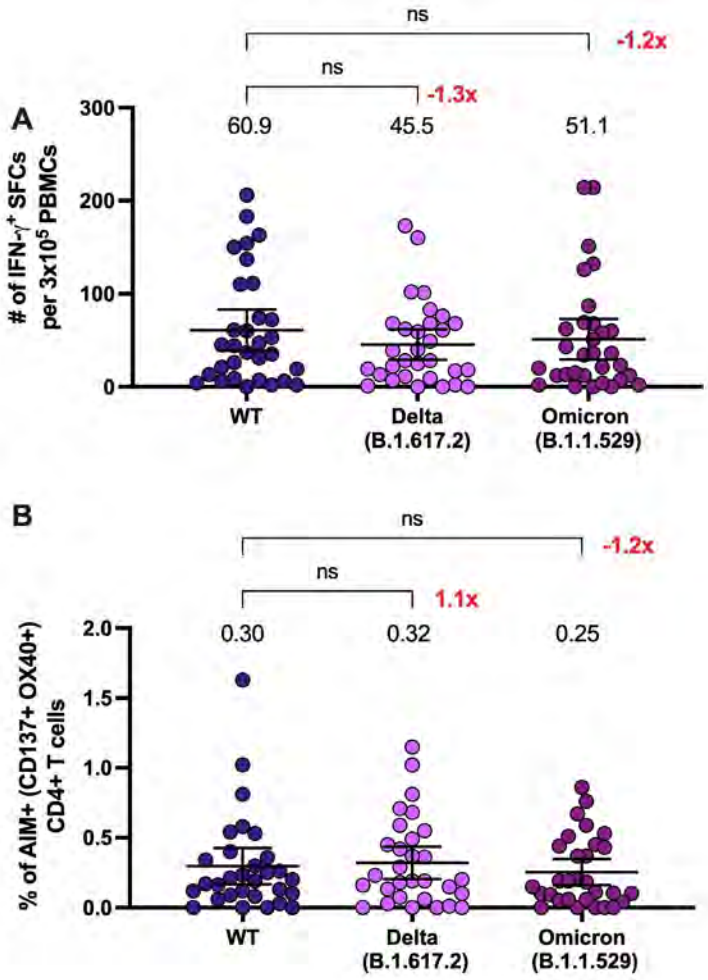

A
****
-4.8x
***
-1.5x
241.8
159.2
50.7
10000
1000
100
10
1
Reciprocal antibody titer against pseudoty ped virus (ID80)
D614G
Delta (B.1.617.2)
Omicron (B.1.1.529)
B
100
93
76.7
100
80
60
40
20
0
Seropositivity Rate (%) of Neutralizing antibodies
D614G
Delta (B.1.617.2)
Omicron (B.1.1.529)

Figure 5


A
# of IFN-γ+ SFCs per 3x10^5 PBMCs
300
200
100
0
ns
-1.2x
ns
-1.3x
60.9
45.5
51.1
WT
Delta (B.1.617.2)
Omicron (B.1.1.529)
B
% of AIM+ (CD137+ OX40+) CD4+ T cells
2.0
1.5
1.0
0.5
0.0
ns
-1.2x
ns
1.1x
0.30
0.32
0.25
WT
Delta (B.1.617.2)
Omicron (B.1.1.529)

2.3. Estudo chinês mostra efetividade da CoronaVac contra casos graves da variante delta

Uma pesquisa publicada na revista científica Annals of Internal Medicine mostrou que vacinas de vírus inativado como a CoronaVac apresentam alta efetividade para combater a variante delta do SARS-CoV-2, protegendo contra casos graves durante a circulação da delta entre maio e junho de 2021 em Guangdong, na China.

Os cientistas chineses avaliaram 10.805 pacientes adultos que foram diagnosticados com Covid-19, divididos em três grupos: não vacinados, vacinados com apenas uma dose e totalmente imunizados (duas doses) com as vacinas de vírus inativado mais usadas na China – CoronaVac (aplicada em cerca de 60% dos participantes) e HB02/Sinopharm (aplicada em cerca de 40%). Em seguida, estimaram a efetividade dos imunizantes contra a infecção, contra casos sintomáticos, contra pneumonia e contra a doença grave.

Em indivíduos com esquema vacinal completo, a efetividade foi de 52% contra infecções, 60% contra casos sintomáticos, 78% contra pneumonia e 100% contra casos severos de Covid-19. Já entre os parcialmente imunizados, as vacinas forneceram uma proteção de 10,7% contra infecções, 6,8% contra casos sintomáticos e 11,6% contra pneumonia.

Os resultados evidenciam a eficácia no mundo real de vacinas de vírus inativado, confirmando os achados de outros estudos de efetividade já publicados, como o Projeto S do Butantan e uma pesquisa chilena com dez milhões de pessoas, que avaliaram a CoronaVac. "Além disso, a pesquisa reforça a necessidade das duas doses, mostrando que a vacinação parcial não confere proteção suficiente", apontam os autores do artigo.

Os pesquisadores destacam que vacinas de vírus inativado são as melhores candidatas para imunização em países em desenvolvimento, já que são fáceis de transportar e não exigem armazenamento em congeladores. Mais de dois bilhões de doses de CoronaVac já foram aplicadas em 45 países.

Publicado em: 1/02/2022

Annals of Internal Medicine

ORIGINAL RESEARCH

Effectiveness of Inactivated COVID-19 Vaccines Against Illness Caused by the B.1.617.2 (Delta) Variant During an Outbreak in Guangdong, China

A Cohort Study

Min Kang, MMed*; Yao Yi, MMed*; Yan Li, PhD; Limei Sun, BM; Aiping Deng, MMed; Ting Hu, MMed; Jiayi Zhang, MMed; Jun Liu, MPAM; Mingji Cheng, MMed; Shen Xie, MMed; Min Luo, MMed; Jing Jiang, MMed; Yawen Jiang, PhD; Shixing Tang, PhD; and Jianfeng He, BSc

Background: Real-world evidence on inactivated COVID-19 vaccines against the highly transmissible B.1.617.2 (Delta) variant of SARS-CoV-2 is limited, leaving an important gap in the evidence base about inactivated COVID-19 vaccines for use by immunization programs.

Objective: To estimate inactivated vaccine effectiveness (VE) against the B.1.617.2 variant.

Design: Retrospective cohort study.

Setting: The study was based on the first outbreak of the B.1.617.2 variant in mainland China that was discovered and traced in Guangdong in May and June 2021.

Participants: 10 805 adult case patients with laboratory-confirmed infection and close contacts.

Measurements: Participants were categorized as unvaccinated, partially vaccinated (1 dose), and fully vaccinated (2 doses). We estimated VE against the primary outcome of pneumonia and the secondary outcomes of infections, symptomatic infections, and severe or critical illness associated with the B.1.617.2 variant.

Results: Results are reported in the order of outcome severity. Of 10 805 participants, 1.3% contracted infections, 1.2% developed symptomatic infections, 1.1% had pneumonia, and 0.2% had severe or critical illness. The adjusted VEs of full vaccination were 51.8% (95% CI, 20.3% to 83.2%) against infection, 60.4% (CI, 31.8% to 88.9%) against symptomatic infection, and 78.4% (CI, 56.9% to 99.9%) against pneumonia. Also, full vaccination was 100% (CI, 98.4% to 100.0%) effective against severe or critical illness. By contrast, the adjusted VEs of partial vaccination against infection, symptomatic infection, and pneumonia were 10.7% (CI, −41.2% to 62.6%), 6.8% (CI, −47.4% to 61.0%), and 11.6% (CI, −42.6% to 65.8%), respectively.

Limitation: Observational study with possible unmeasured confounders; insufficient data to do reliable subgroup analyses by age and vaccine brand.

Conclusion: Full vaccination with inactivated vaccines is effective against the B.1.617.2 variant. Effort should be made to ensure full vaccination of target populations.

Primary Funding Source: National Natural Science Foundation of China and Key-Area Research and Development Program of Guangdong Province.

Ann Intern Med. doi:10.7326/M21-3509 Annals.org
For author, article, and disclosure information, see end of text.
This article was published at Annals.org on 1 February 2022.
* Min Kang and Yao Yi contributed equally to this work.

Vaccination is considered an indispensable part of the long-term management of the COVID-19 pandemic (1, 2). Because of an unprecedented global effort to develop COVID-19 vaccines, numerous types of vaccines were approved in many jurisdictions by early 2021 (2–4). Among these, several were developed using whole-virus inactivation technology and have received partial or full approval in China and many other countries (4–7). In China alone, 4 inactivated vaccines have been distributed and administered: HB02 (Sinopharm), WIV04 (Sinopharm), CoronaVac (Sinovac), and BICV (Biokangtai), among which HB02 and CoronaVac were used most frequently (4, 8). Because of their long shelf life without the need for ultracold chain storage, inactivated vaccines are relatively easy to store and dispense (9–11). Combined with their documented efficacy from randomized clinical trials (RCTs), this may make inactivated vaccines a near-ideal candidate for mass immunization programs in low- and middle-income countries (8, 9, 12).

Although RCTs are the gold standard to estimate efficacy, their results may have limited generalizability because of participant selection and exclusion criteria and implementation restrictions. Real-world evidence supplements RCT data by providing insight on comparative effectiveness in various situations: among populations excluded from or insufficiently included in licensure RCTs, under different settings and epidemiologic situations, using alternative outcomes, or comparing a different lineage of the pathogen (13, 14). To date, published real-world evidence on COVID-19 vaccines has largely focused on messenger RNA (mRNA) vaccines, and these findings are similar to corresponding RCT results (15–19). Real-world evidence on inactivated vaccines remains sparse. One study in Chile assessed the effectiveness of CoronaVac, an inactivated vaccine used for mass vaccination in more than 20 countries, and provided convincing evidence of its protective effect against COVID-19 (20).

Owing to the effectively implemented zero-infection strategy, China has managed to clear all sporadic local outbreaks since April 2020, most of which lasted for less than 3 weeks and infected fewer than 100 persons. In late May 2021, an outbreak of a highly transmissible variant of SARS-CoV-2, the B.1.617.2 (Delta) variant, was discovered and traced in Guangdong, China (21). Characterized by

protein mutations T19R, Δ157-158, L452R, T478K, G, P681R, and D950N, the B.1.617.2 variant repro- s at a faster rate than previous lineages seen in a, posing substantial challenges for disease control 2). This was the first outbreak of the B.1.617.2 variant inland China. It lasted from 21 May to 18 June 2021, g which time 167 persons infected with the Delta var- were identified in clinical settings, in quarantine, or gh community screenings. In addition to case identifi- n, contact tracing of the outbreak continued through ne 2021, after which no more cases were reported. e the start of this outbreak, China had already started pidly roll out mass immunization campaigns, and gdong province was one of the forerunners of vaccine yment. Specifically, more than 90 million doses of ivated vaccine were administered in Guangdong re mid-June 2021. As such, the outbreak was an rtunity to gain insight into the effectiveness of ivated vaccines against the B.1.617.2 variant.

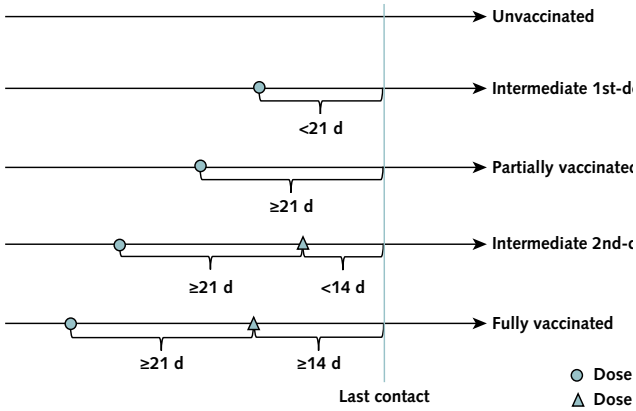
y analyzing vaccination, surveillance, screening, trac- nd quarantine data on China's COVID-19 prevention control, we could assess the real-world effectiveness activated vaccines against COVID-19 caused by the 17.2 variant. More than 2 billion doses of inactivated D-19 vaccine have been administered in more than untries and regions. Thus, evidence on the effective- of inactivated vaccines against the rampantly ing variant is critical for public health agencies and nunities globally.

THODS

y Population and Design

he local outbreak in Guangdong was started by an rted infection from abroad; that patient transmitted a local resident, who was the index case patient. All ndary local cases were well traced and linked to the case in a single long chain of transmission (23, 24). cordance with national and provincial protocols for D-19 prevention and control, close contacts were ed as all people who lived in the same household ayed in the same public space without protection n close proximity in the 4 days before illness onset mptomatic cases or sampling of the first positive men for asymptomatic cases (25). All close contacts traced, mandatorily quarantined in centralized man- facilities, and followed with multiple reverse tran- ase polymerase chain reaction tests; they became of our study cohort as the outbreak was proceeding being managed. The Close Contacts Management on of the **Appendix** (available at Annals.org) gives ional information on close contact definition and gement, and the Laboratory Confirmation section information on specifications of test kits. Of note, all patients were themselves close contacts of their eam cases before they became infected. Therefore, ase patients and their close contacts made up a

Figure 1. Vaccination status definitions.



individuals and their close contacts identified in Guangdong outbreak.

In addition to the index case (the first local infect health authorities identified 12 500 individuals, inclu secondary case patients and close contacts. All pos specimens were subject to whole-genome sequenc Individuals were excluded if basic demographic informa was missing or if they received noninactivated vacci Because immunization campaigns in China requeste 21-day interval after the first dose and COVID-19 vacc were provided only to adults until July 2021, persons received 2 doses of vaccine but less than 21 days apa were younger than 18 years were also excluded.

This study was approved by the institutional et committee of the Guangdong Provincial Center Disease Control and Prevention. The data in the st were collected per administrative requirements disease control and surveillance and were anonymi for analysis. Participants were informed about the req ments of disease surveillance and provided oral cons

Vaccination Status

To determine vaccination status, we used the num- ber of doses received and time elapsed since the most recent dose. On the basis of vaccination electronic records, participants were categorized into an unvacci- nated group, a partially vaccinated (1-dose) group, a fully vaccinated (2-dose) group. The unvaccinated gr consisted of persons who did not receive any COVID vaccines before their last known contact with a confir case patient. The partially vaccinated group compr those who received their first dose 21 days or m before the last known contact. Persons who received their second dose at least 14 days before the last known contact made up the fully vaccinated group. Our prin analysis was a 3-group comparison. Those who rece their first dose within 21 days (intermediate first dose their second dose within 14 days (intermediate sec

:omes

he primary outcome was pneumonia caused by the 17.2 variant of SARS-CoV-2. Secondary outcomes infections, symptomatic infections, and severe or al illness associated with the B.1.617.2 variant. ever, results are reported following the hierarchy of ome severity. Symptoms and severity were defined rding to China's Diagnosis and Treatment Protocol OVID-19 Patients (26). Pneumonia was diagnosed chest imaging characteristics. Severe cases were in which the patient had a respiratory rate above eaths/min, resting blood oxygen saturation of 93% wer, or Pa_{O_2}–Fl_{O_2} ratio of 300 mm Hg or lower (26). tical cases, patients had respiratory failure leading echanical ventilation, experienced shock, or sus- d any other organ failure that required intensive (26). Severity was based on a participant's most seri- nanifestations during the follow-up period.

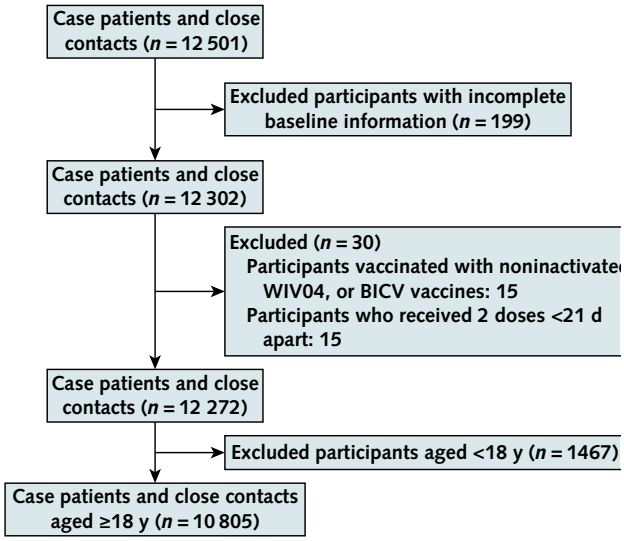
:acteristics and Covariates

pidemiologic investigators collected sociodemo- hic information, including age, sex, address, occu- n, and contact frequency. These variables could ntially confound the vaccine effectiveness (VE) esti- s by correlating with both vaccination and outcomes were used as covariates in subsequent analyses. Age categorized as 18 to 34 years, 35 to 49 years, or 50 or older. In adherence to the national prevention control scheme, investigators adjudicated contact fre- cy as occasionally, sometimes, or frequently. Contact ency might correlate with vaccination status because nated persons could be tempted to reduce adher- to nonpharmaceutical measures, such as social dis- ng (27, 28). Occupation might have been associated vaccination status, in that professionals in occupa- with high exposure risk were granted priority for nation during early 2021. To control potential con- ding due to cross-occupation heterogeneity in the ces of vaccination and exposure to the virus during l interaction, we created indicators of working in res- nt services, working as a health care provider, and currently unemployed. In addition, geographic might lead to bias in estimation of VE if left unad- d for because areas with different intensity of trans- on might also have had different access to vaccines. ifically, 2 subdistricts in Guangzhou (subdistricts A 3 for simplicity) were epicenters of the outbreak. The in these 2 communities accounted for more than of all outbreak cases. As such, residents of these 2 istricts could have had higher risk for exposure, yet ss to vaccines in these communities was not neces- the same as in other places. Therefore, an indicator created for each of the 2 epicenter subdistricts and as a covariate in addition to the sociodemographic bles.

istical Analysis

ary Analysis

Figure 2. Study flow diagram.



outcome was calculated in reference to the unvaccina group and subtracted from 1. In addition, we used m variable logistic regressions to account for covariates could potentially confound effect estimates. To estin adjusted VE (aVE) from multivariable logistic regressi we first calculated the adjusted RR (aRR) that equaled the ratio of the predicted event probability in each va nation group to that in the unvaccinated group; the Adjusted Risk Ratio section of the **Appendix** elabor on this (29–31). The aVE was then calculated as 1 − a We used aRRs to calculate aVEs because RRs are intui- tively understandable for cohort studies and because odds ratios consistently underestimated RRs for prc tion effects, leading to potentially exaggerated VE esti- mates (32). The SEs of aRRs were estimated using the delta method, which is frequently used for nonlinear transformations of regression coefficients (33). We u Stata, version 16 (StataCorp), with the logit routine and its postestimation features for analyses.

Sensitivity Analysis

In a prespecified sensitivity analysis, vaccination st was based on each person's number of doses before outbreak. In this analysis, anyone who received their dose but not their second dose before 7 May 2021 days before 21 May 2021) was assigned to the part vaccinated group, whereas those who received t doses before 7 May 2021 made up the fully vaccina group. Those who received the initial dose after 7 2021 were excluded from this analysis. In additio between-dose window was not considered when de mining vaccination status.

We also did several post hoc sensitivity analyses, r ing 1 change to the base case at a time (Post Hoc Sensit Analyses section of the **Appendix**). Specifically, we inclu all vaccination statuses as distinct exposure groups, u

Table 1. Participant Characteristics, by Vaccination Status*

Characteristic	Unvaccinated (*n* = 5888 [54.5%])	Intermediate 1st Dose (*n* = 2286 [21.1%])	Partially Vaccinated (*n* = 841 [7.8%])	Intermediate 2nd Dose (*n* = 387 [3.6%])	Fully Vaccinated (*n* = 1403 [13.0%])	Total (*n* = 10 805)
Sex						
Male	3174 (53.9)	1197 (52.4)	452 (53.7)	188 (48.6)	769 (54.8)	5780 (53.5)
Female	2714 (46.1)	1089 (47.6)	389 (46.3)	199 (51.4)	634 (45.2)	5025 (46.5)
Mean age (SD), y	48.0 (18.1)	38.3 (11.4)	38.5 (10.9)	38.8 (10.7)	39.3 (10.5)	43.8 (16.0)
Age group						
18–34 y	1798 (30.5)	967 (42.3)	335 (39.8)	154 (39.8)	510 (36.4)	3764 (34.8)
35–49 y	1357 (23.0)	876 (38.3)	339 (40.3)	159 (41.1)	608 (43.3)	3339 (30.9)
≥50 y	2733 (46.4)	443 (19.4)	167 (19.9)	74 (19.1)	285 (20.3)	3702 (34.3)
Contact frequency						
Occasionally	2438 (41.4)	880 (38.5)	325 (38.6)	133 (34.4)	494 (35.2)	4270 (39.5)
Sometimes	3294 (55.9)	1342 (58.7)	473 (56.2)	234 (60.5)	827 (58.9)	6170 (57.1)
Frequently	156 (2.7)	64 (2.8)	43 (5.1)	20 (5.2)	82 (5.8)	365 (3.4)
Subdistrict						
A	148 (2.5)	44 (1.9)	47 (5.6)	7 (1.8)	45 (3.2)	291 (2.7)
B	806 (13.7)	130 (5.7)	81 (9.6)	30 (7.8)	141 (10.0)	1188 (11.0)
Other	4934 (83.8)	2112 (92.4)	713 (84.8)	350 (90.4)	1217 (86.7)	9326 (86.3)
Occupation						
Restaurant services	225 (3.8)	186 (8.1)	48 (5.7)	22 (5.7)	34 (2.4)	515 (4.8)
Unemployed/home	182 (3.1)	60 (2.6)	27 (3.2)	7 (1.8)	27 (1.9)	303 (2.8)
Health care worker	32 (0.5)	25 (1.1)	12 (1.4)	11 (2.8)	141 (10.0)	221 (2.0)
Other	5449 (92.5)	2015 (88.1)	754 (89.7)	347 (89.7)	1201 (85.6)	9766 (90.4)

* Values are numbers (percentages) unless otherwise specified.

potential effect of unmeasured confounders, we computed the E-values (E-Value section of the **Appendix**), which are the minimum strengths of association, on the RR scale, that unmeasured confounders would need to have with both the vaccination status and the outcomes to fully explain away a specific vaccination status–outcome association, conditional on the measured covariates (34).

Role of the Funding Source

The National Natural Science Foundation of China and Key-Area Research and Development Program of Guangdong Province had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

RESULTS

We applied the inclusion and exclusion criteria to the 12 501 cases and close contacts that were eligible for initial inclusion. Among these, 199 persons (1.6%) had missing sociodemographic information, 7 (0.1%) were vaccinated with noninactivated vaccines, 8 (0.1%) were vaccinated with WIV04 or BICV vaccines (excluded because of limited sample sizes), 15 (0.1%) had received 2 doses less than 21 days apart, and 1467 (11.7%) were younger than 18 years. Consequently, 10 805 participants met all inclusion and no exclusion criteria, none of whom had been previously infected with SARS-CoV-2. The participants were grouped into 5 categories based on vaccination history. **Figure 2** shows the selection flow chart.

Of the 10 805 persons who met inclusion but not exclusion criteria, 5888 (54.5%) were unvaccinated, 2286 (21.1%) had an intermediate first dose, 841 (7.8%) were partially vaccinated, 387 (3.6%) had an intermediate second dose, and 1403 (13.0%) were fully vaccinated (**Table 1**). **Appendix Table 1** (available at Annals.org) shows the distribution of the vaccine brands among vaccinated participants.

Across the 5 groups, age, contact frequency, living in subdistrict A or B, and occupation were unbalanced, whereas sex was distributed similarly. The unvaccinated group had the greatest mean age (48.0 years), the highest proportion of participants aged 50 years or older (46.4%), the highest proportion of occasional contact (41.4%), and the lowest proportion of frequent contact (2.7%). In addition, the unvaccinated group had a higher percentage of subdistrict B residents (13.7%) than any other group, whereas its percentage of subdistrict A residents (2.5%) was lower than that of the partially and fully vaccinated groups, but not of the intermediate first-dose and second-dose groups. The unvaccinated group had a proportion of unemployed participants (3.1%) second only to that of the partially vaccinated group and had the second-lowest proportion of restaurant services professionals (3.8%)—surpassed only by the fully vaccinated group. **Table 1** lists the characteristics of the groups, and **Table 2** summarizes the outcomes.

Unadjusted VE estimates are shown in **Table 3**. In the unvaccinated, partially vaccinated, and fully vaccinated groups, 93 (1.6%), 13 (1.5%), and 10 (0.7%) persons, respectively, had infections, corresponding to RRs of 0.979 (95% CI, 0.415 to 1.542) in the partially vaccinated group and 0.451 (CI, 0.158 to 0.744) in the fully vaccinated group. Accordingly, the unadjusted VEs of partial

Table 2. Outcomes, by Vaccination Status*

Outcome	Unvaccinated (*n* = 5888 [54.5%])	Intermediate 1st Dose (*n* = 2286 [21.1%])	Partially Vaccinated (*n* = 841 [7.8%])	Intermediate 2nd Dose (*n* = 387 [3.6%])	Fully Vaccinated (*n* = 1403 [13.0%])	Total (*n* = 10 805)
Infection						
Yes	93 (1.6)	16 (0.7)	13 (1.5)	4 (1.0)	10 (0.7)	136 (1.3)
No	5795 (98.4)	2270 (99.3)	828 (98.5)	383 (99.0)	1393 (99.3)	10 669 (98.7)
Symptomatic infection						
Yes	92 (1.6)	16 (0.7)	13 (1.5)	4 (1.0)	8 (0.6)	133 (1.2)
No	5796 (98.4)	2270 (99.3)	828 (98.5)	383 (99.0)	1395 (99.4)	10 672 (98.8)
Pneumonia						
Yes	85 (1.4)	16 (0.7)	12 (1.4)	3 (0.8)	4 (0.3)	120 (1.1)
No	5803 (98.6)	2270 (99.3)	829 (98.6)	384 (99.2)	1399 (99.7)	10 685 (98.9)
Severe or critical						
Yes	19 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	19 (0.2)
No	5869 (99.7)	2286 (100.0)	841 (100.0)	387 (100.0)	1403 (100.0)	10 786 (99.8)

* Values are numbers (percentages).

and full vaccination against infection were 2.1% (CI, −54.2% to 58.5%) and 54.9% (CI, 25.6% to 84.2%), respectively. Also, 92 persons (1.6%) in the unvaccinated group, 13 (1.5%) in the partially vaccinated group, and 8 (0.6%) in the fully vaccinated group had symptomatic infections, which amounted to RRs of 0.989 (CI, 0.419 to 1.559) in the partially vaccinated group and 0.365 (CI, 0.102 to 0.628) in the fully vaccinated group. Based on the RR results, the unadjusted VEs against symptomatic infection associated with the B.1.617.2 variant among the partially and fully vaccinated groups were 1.1% (CI, −55.9% to 58.1%) and 63.5% (CI, 37.2% to 89.8%), respectively.

There were 85 cases (1.4%) of COVID-19 pneumonia in the unvaccinated group, 12 (1.4%) in the partially vaccinated group, and 4 (0.3%) in the fully vaccinated group. As such, the RRs of pneumonia associated with partial and full vaccination were 0.988 (CI, 0.395 to 1.582) and 0.197 (CI, 0.000 to 0.395), respectively, which corresponded to unadjusted VEs of 1.2% (CI, −58.2% to 60.5%) and 80.3% (CI, 60.5% to 100.0%) against pneumonia caused by the B.1.617.2 variant.

No severe or critical cases occurred among vaccinated participants. By contrast, unvaccinated participants had 19 (0.3%) severe or critical cases. As such, the unadjusted VEs of partial and full vaccination were 100.0% (CI, 98.5% to 100.0%) and 100.0% (CI, 98.4% to 100.0%), respectively, against severe or critical COVID-19 caused by the B.1.617.2 variant.

The aVEs and aRRs from multivariable logistic regressions are presented in **Table 3** and **Appendix Table 2** (available at Annals.org). The main findings on aVEs are also shown in **Figure 3**. Multivariable analyses of severe or critical cases could not be done. On the basis of aRRs and aVEs, partial vaccination (compared with no vaccination) was not associated with a statistically significant difference in the incidence of any outcome. However, the aRRs of full vaccination against infection (0.482 [CI, 0.168 to 0.797]), symptomatic infection (0.396 [CI, 0.111 to 0.682]), and pneumonia (0.216 [CI, 0.001 to 0.431]) were significant. The corresponding aVEs were 51.8% (CI, 20.3% to 83.2%), 60.4% (CI, 31.8% to 88.9%), and 78.4% (CI, 56.9% to 99.9%).

Appendix Table 3 (available at Annals.org) shows the results of the prespecified sensitivity analyses using an alternative definition for vaccination status. Full vaccination was consistently effective against all outcomes, whereas partial vaccination was not. Also, the results of the post hoc sensitivity analyses (**Appendix Tables 4** to **6**, available at Annals.org) resembled the base-case results. The E-values for the strengths of association between unmeasured confounders and both the vaccination status and outcomes needed to explain away the aRR are listed in **Appendix Table 7** (available at Annals.org) and discussed in the E-Value section of the **Appendix**.

Discussion

Our study evaluated the effectiveness of inactivated COVID-19 vaccines against infections, symptomatic infections, pneumonia, and severe or critical illness caused by the B.1.617.2 variant in a real-world setting. By analyzing the cohort from a single transmission chain, we showed that the VEs of inactivated vaccines against the B.1.617.2 variant were 52% for SARS-CoV-2 infection, 60% for symptomatic COVID-19, 78% for COVID-19 pneumonia, and 100% for severe or critical COVID-19.

Our findings confirm the VE of inactivated vaccines against COVID-19 that has been reported by clinical and real-world studies (8, 12, 20). For example, an RCT in Brazil showed that CoronaVac, one of the inactivated vaccines, was 51% efficacious against symptomatic infections (12). In addition, a real-world study in Chile estimated that the VEs of CoronaVac against symptoms and hospitalizations due to COVID-19 caused by early lineages of SARS-CoV-2 were 66% and 88%, respectively (20). More, our findings confirm that inactivated COVID-19 vaccines will be effective even when the B.1.617.2 variant is prevalent, echoing recent findings on the effectiveness of mRNA-based vaccines against illness caused by that variant (22). However, inactivated vaccines may

Table 3. VE in Preventing Infections, Symptomatic Infections, Pneumonia, and Severe or Critical Cases, by Vaccination Status

Outcome and Vaccination Status	Events/Participants, *n/N* (% [95% CI])	Unadjusted		Adjusted*	
		RR (95% CI)	VE (95% CI), %	aRR (95% CI)	aVE (95% CI), %
Infection					
Unvaccinated	93/5888 (1.6 [1.3 to 1.9])	Reference	-	-	-
Partially vaccinated	13/841 (1.5 [0.8 to 2.6])	0.979 (0.415 to 1.542)	2.1 (−54.2 to 58.5)	0.893 (0.374 to 1.412)	10.7 (−41.2 to 62.6)
Fully vaccinated	10/1403 (0.7 [0.3 to 1.3])	0.451 (0.158 to 0.744)	54.9 (25.6 to 84.2)	0.482 (0.168 to 0.797)	51.8 (20.3 to 83.2)
Symptomatic infection					
Unvaccinated	92/5888 (1.6 [1.3 to 1.9])	Reference	-	-	-
Partially vaccinated	13/841 (1.5 [0.8 to 2.6])	0.989 (0.419 to 1.559)	1.1 (−55.9 to 58.1)	0.932 (0.390 to 1.474)	6.8 (−47.4 to 61.0)
Fully vaccinated	8/1403 (0.6 [0.2 to 1.1])	0.365 (0.102 to 0.628)	63.5 (37.2 to 89.8)	0.396 (0.111 to 0.682)	60.4 (31.8 to 88.9)
Pneumonia					
Unvaccinated	85/5888 (1.4 [1.2 to 1.8])	Reference	-	-	-
Partially vaccinated	12/841 (1.4 [0.7 to 2.5])	0.988 (0.395 to 1.582)	1.2 (−58.2 to 60.5)	0.884 (0.342 to 1.426)	11.6 (−42.6 to 65.8)
Fully vaccinated	4/1403 (0.3 [0.1 to 0.7])	0.197 (0.000 to 0.395)	80.3 (60.5 to 100.0)	0.216 (0.001 to 0.431)	78.4 (56.9 to 99.9)
Severe or critical					
Unvaccinated	19/5888 (0.3 [0.2 to 0.5])	Reference	-	-	-
Partially vaccinated	0/841 (0.0 [0.0 to 0.4])	0.000 (0.000 to 0.015)	100.0 (98.5 to 100.0)†	-	-
Fully vaccinated	0/1403 (0.0 [0.0 to 0.3])	0.000 (0.000 to 0.016)	100.0 (98.4 to 100.0)†	-	-

aRR = adjusted risk ratio; aVE = adjusted vaccine effectiveness; RR = risk ratio; VE = vaccine effectiveness.
* Adjusted for sex, age, occupation, subdistrict, and contact frequency.
† The section Estimation of CIs for Groups With Zero-Event Cells in the Appendix (available at Annals.org) presents the methods of estimating the 95% CIs of the VE of preventing severe or critical cases.

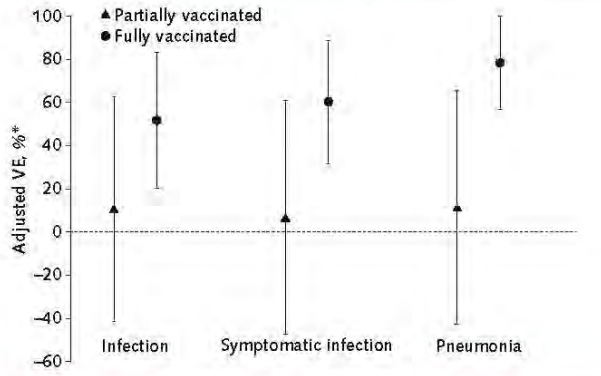
not be equally effective against the B.1.617.2 and other variants—a pattern shared by other vaccines (20, 22). Of note, the effect sizes of vaccines from the present study were not necessarily outstanding compared with reports in the literature (22, 35, 36).

CoronaVac and HB02 are both authorized by the World Health Organization for emergency use and together accounted for almost half of the COVID-19 vaccine doses dispensed globally as of October 2021 (37, 38). Therefore, our study has important policy implications. First, it is critically important to continue mass immunization programs to ensure full vaccination of the target population. As indicated by the results, partial vaccination with inactivated vaccines provides insufficient protection. Second, inactivated vaccines are a viable option to prevent COVID-19 despite recent mutations of the virus. Third, the estimates of VE against infections and illness call for refreshed evaluations of strategies to manage the pandemic in the long term. For example, preventing symptomatic infections remains an important task of global public health efforts because symptomatic patients are the ones suffering from illness and requiring medical attention. Fourth, although the current findings on VE are encouraging, recent reports on immunity waning are alarming, such that booster shots may be warranted (38, 39). Given their real-world effectiveness as well as convenient stocking and distribution, inactivated vaccines should be considered an option for immunity reinforcement programs on completion of population-level, 2-dose vaccination.

To our knowledge, this study adds unique contributions to the scientific literature. First, it expanded on a previous study on the real-word effectiveness of inactivated vaccines by investigating 2 instead of 1 specific type of vaccine in this class (20). Second, it provided preliminary evidence of the VE of inactivated vaccines against the B.1.617.2 variant using a cohort study design. Third, it is the first study that documented the effectiveness of COVID-19 vaccines against clinical outcomes other than intermediate end points of COVID-19 in mainland China using a relatively large sample size. By combining these features, the present study generated new evidence that helps informed decision making in regions that heavily engage inactivated vaccines to combat the pandemic, such as Southeast Asia and Latin America. A caveat for the interpretation of results is that the VE estimates may not necessarily apply equally to both brands of inactivated vaccines.

Our study has limitations. First, as with all observational studies, and although we controlled for known covariates, residual unmeasured confounders might have compromised the validity of the analyses. Second, moderate incidence rates and vaccination rates undermined the feasibility of subgroup analyses. For example, only 6 persons aged 60 years or older were fully vaccinated because the priority target group during the initial rollout was those aged 18 to 59 years; this makes reliable subgroup analyses by age impossible. A related concern was that the precision of the estimates, which were dependent on subgroup sample sizes and the number of infections, was suboptimal as reflected by the wide CIs. Third, although hospitalization is a routinely used outcome in the evaluation of VE, we did not use it because all patients with COVID-19 were hospitalized in China regardless of severity. In the present study, the outcome of severe or critical illness was used in lieu of hospitalization. Despite these limitations, we believe that our study provides useful insights on the effectiveness of vaccines and suggests that full vaccination with inactivated vaccines

Figure 3. Adjusted VE against outcomes of different severity associated with the B.1.617.2 variant, by vaccination status.



VE = vaccine effectiveness.
* Adjusted for sex, age, occupation, subdistrict, and contact frequency.

may be effective against COVID-19 associated with the B.1.617.2 variant of SARS-CoV-2.

From School of Public Health, Southern Medical University, and Guangdong Provincial Center for Disease Control and Prevention, Guangzhou, China (M.K.); Guangdong Provincial Center for Disease Control and Prevention, Guangzhou, China (Y.Y., Y.L., L.S., A.D., T.H., J.Z., J.L., M.C., S.X., M.L., J.J., J.H.); School of Public Health (Shenzhen), Sun Yat-sen University, Shenzhen, China (Y.J.); and School of Public Health, Southern Medical University, Guangzhou, China (S.T.).

Acknowledgment: The authors thank Zhongjie Li, Zhijie An, Dan Wu, Ying Qin, Qianqian Liu, and Jing Wu for their valuable guidance and suggestions.

Grant Support: By grant 82041030 from the National Natural Science Foundation of China and grant 2019B111103001 from the Key-Area Research and Development Program of Guangdong Province.

Disclosures: Authors have reported no disclosures of interest. Forms can be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M21-3509.

Reproducible Research Statement: *Study protocol:* Not available. *Statistical code:* Available from Dr. Jiang (e-mail, jiangyw26@mail.sysu.edu.cn) on reasonable request. *Data set:* Available from Mr. Kang (e-mail, kangmin@yeah.net) on reasonable request.

Corresponding Authors: Yawen Jiang, PhD, Room 215, Mingde Garden #6, Sun Yat-sen University, 132 East Outer Ring Road, Panyu District, Guangzhou, China (e-mail, jiangyw26@mail.sysu.edu.cn); Shixing Tang, PhD, No. 1023-1063, Shatai South Road, Baiyun District, Guangzhou, China (e-mail, tamgshixing@smu.edu.cn); and Jianfeng He, BSc, No. 160, Qunxian Road, Panyu District, Guangzhou, China (e-mail, 1460035443@qq.com).

Author contributions are available at Annals.org.

Previous Posting: This manuscript was posted as a preprint on SSRN on 5 August 2021. doi:10.2139/ssrn.3895639

References

1. Griffin M, Sohrabi C, Alsafi Z, et al. Preparing for COVID-19 exit strategies. Ann Med Surg (Lond). 2021;61:88-92. [PMID: 33391762] doi:10.1016/j.amsu.2020.12.012
2. Moore S, Hill EM, Tildesley MJ, et al. Vaccination and non-pharmaceutical interventions for COVID-19: a mathematical modelling study. Lancet Infect Dis. 2021;21:793-802. [PMID: 33743847] doi:10.1016/S1473-3099(21)00143-2
3. Forni G, Mantovani A; COVID-19 Commission of Accademia Nazionale dei Lincei, Rome. COVID-19 vaccines: where we stand and challenges ahead. Cell Death Differ. 2021;28:626-639. [PMID: 33479399] doi:10.1038/s41418-020-00720-9
4. Baraniuk C. What do we know about China's covid-19 vaccines. BMJ. 2021;373:n912. [PMID: 33836994] doi:10.1136/bmj.n912
5. Baraniuk C. Covid-19: What do we know about Sputnik V and other Russian vaccines. BMJ. 2021;372:n743. [PMID: 33741559] doi:10.1136/bmj.n743
6. Thiagarajan K. What do we know about India's Covaxin vaccine. BMJ. 2021;373:n997. [PMID: 33879478] doi:10.1136/bmj.n997
7. Liu R, Shen M, Kerry F. Kangtai Biological's COVID-19 vaccine gets emergency use approval in China. Reuters. 14 May 2021. Accessed at www.reuters.com/article/us-health-coronavirus-vaccine-kangtai-idUSKBN2CV1F6 on 27 June 2021.
8. Al Kaabi N, Zhang Y, Xia S, et al. Effect of 2 inactivated SARS-CoV-2 vaccines on symptomatic COVID-19 infection in adults: a randomized clinical trial. JAMA. 2021;326:35-45. [PMID: 34037666] doi:10.1001/jama.2021.8565
9. Rostad CA, Anderson EJ. Optimism and caution for an inactivated COVID-19 vaccine. Lancet Infect Dis. 2021;21:581-582. [PMID: 33485467] doi:10.1016/S1473-3099(20)30988-9
10. Mallapaty S. China COVID vaccine reports mixed results – what does that mean for the pandemic. Nature. 2021. [PMID: 33452510] doi:10.1038/d41586-021-00094-z
11. Holm MR, Poland GA. Critical aspects of packaging, storage, preparation, and administration of mRNA and adenovirus-vectored COVID-19 vaccines for optimal efficacy [Editorial]. Vaccine. 2021;39:457-459. [PMID: 33339671] doi:10.1016/j.vaccine.2020.12.017
12. Palacios R, Batista AP, Albuquerque CSN, et al. Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in Brazil: the PROFISCOV study. SSRN. Preprint posted online 14 April 2021. doi:10.2139/ssrn.3822780
13. Berger ML, Sox H, Willke RJ, et al. Good practices for real-world data studies of treatment and/or comparative effectiveness: recommendations from the joint ISPOR-ISPE Special Task Force on real-world evidence in health care decision making. Pharmacoepidemiol Drug Saf. 2017;26:1033-1039. [PMID: 28913966] doi:10.1002/pds.4297
14. Bartlett VL, Dhruva SS, Shah ND, et al. Feasibility of using real-world data to replicate clinical trial evidence. JAMA Netw Open. 2019;2:e1912869. [PMID: 31596493] doi:10.1001/jamanetworkopen.2019.12869
15. Haas EJ, Angulo FJ, McLaughlin JM, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. Lancet. 2021;397:1819-1829. [PMID: 33964222] doi:10.1016/S0140-6736(21)00947-8
16. Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA Covid-19 vaccine in a nationwide mass vaccination setting. N Engl J Med. 2021;384:1412-1423. [PMID: 33626250] doi:10.1056/NEJMoa2101765
17. Swift MD, Breeher LE, Tande AJ, et al. Effectiveness of messenger RNA coronavirus disease 2019 (COVID-19) vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

infection in a cohort of healthcare personnel. Clin Infect Dis. 2021;73:e1376-e1379. [PMID: 33900384] doi:10.1093/cid/ciab361
18. Pilishvili T, Fleming-Dutra KE, Farrar JL, et al; Vaccine Effectiveness Among Healthcare Personnel Study Team. Interim estimates of vaccine effectiveness of Pfizer-BioNTech and Moderna COVID-19 vaccines among health care personnel – 33 U.S. sites, January-March 2021. MMWR Morb Mortal Wkly Rep. 2021;70:753-758. [PMID: 34014909] doi:10.15585/mmwr.mm7020e2
19. Thompson MG, Burgess JL, Naleway AL, et al. Prevention and attenuation of Covid-19 with the BNT162b2 and mRNA-1273 Vaccines. N Engl J Med. 2021;385:320-329. [PMID: 34192428] doi:10.1056/NEJMoa2107058
20. Jara A, Undurraga EA, González C, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. N Engl J Med. 2021;385:875-884. [PMID: 34233097] doi:10.1056/NEJMoa2107715
21. Campbell F, Archer B, Laurenson-Schafer H, et al. Increased transmissibility and global spread of SARS-CoV-2 variants of concern as at June 2021. Euro Surveill. 2021;26. [PMID: 34142653] doi:10.2807/1560-7917.ES.2021.26.24.2100509
22. Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of Covid-19 vaccines against the B.1.617.2 (delta) variant. N Engl J Med. 2021;385:585-594. [PMID: 34289274] doi:10.1056/NEJMoa2108891
23. Wang Y, Chen R, Hu F, et al. Transmission, viral kinetics and clinical characteristics of the emergent SARS-CoV-2 Delta VOC in Guangzhou, China. EClinicalMedicine. 2021;40:101129. [PMID: 34541481] doi:10.1016/j.eclinm.2021.101129
24. Zhang M, Xiao J, Deng A, et al. Transmission dynamics of an outbreak of the COVID-19 Delta variant B.1.617.2 – Guangdong Province, China, May-June 2021. China CDC Wkly. 2021;3:584-586. [PMID: 34594941] doi:10.46234/ccdcw2021.148
25. National Health Commission of the People's Republic of China. The Prevention and Control Scheme of COVID-19. 8th ed. In Chinese. 14 May 2021. Accessed at www.nhc.gov.cn/jkj/s3577/202105/6f1e8ec6c4a540d99fafef52fc86d0f8/files/4a860a7e85d14d55a22fbab0bbe77cd9.pdf on 6 June 2021.
26. The General Office of National Health Commission of China, National Traditional Chinese Medicine Administration. Diagnosis and treatment protocol for COVID-19 patients (tentative 8th edition). Infectious Diseases & Immunity. 2021;1:8-16. doi:10.1097/01.ID9.0000733564.2178
27. Trogen B, Caplan A. Risk compensation and COVID-19 vaccines [Editorial]. Ann Intern Med. 2021;174:858-859. [PMID: 33646837] doi:10.7326/M20-8251
28. Andersson O, Campos-Mercade P, Meier AN, et al. Anticipation of COVID-19 vaccines reduces willingness to socially distance. J Health Econ. 2021;80:102530. [PMID: 34563830] doi:10.1016/j.jhealeco.2021.102530
29. Norton EC, Miller MM, Kleinman LC. Computing adjusted risk ratios and risk differences in Stata. Stata J. 2013;13:492-509. doi:10.22004/ag.econ.249804
30. Kleinman LC, Norton EC. What's the risk? A simple approach for estimating adjusted risk measures from nonlinear models including logistic regression. Health Serv Res. 2009;44:288-302. [PMID: 18793213] doi:10.1111/j.1475-6773.2008.00900.x
31. Cummings P. Methods for estimating adjusted risk ratios. Stata J. 2009;9:175-96. doi:10.1177/1536867x0900900201
32. Davies HT, Crombie IK, Tavakoli M. When can odds ratios mislead. BMJ. 1998;316:989-91. [PMID: 9550961]
33. Cox C. Delta method. In: Armitage P, Colton T, eds. Encyclopedia of Biostatistics. 2nd ed. J Wiley; 2005:1550-2.
34. VanderWeele TJ, Ding P. Sensitivity analysis in observational research: introducing the E-value. Ann Intern Med. 2017;167:268-274. [PMID: 28693043] doi:10.7326/M16-2607
35. Fowlkes A, Gaglani M, Groover K, et al; HEROES-RECOVER Cohorts. Effectiveness of COVID-19 vaccines in preventing SARS-CoV-2 infection among frontline workers before and during B.1.617.2 (Delta) variant predominance – eight U.S. locations, December 2020-August 2021. MMWR Morb Mortal Wkly Rep. 2021;70:1167-1169. [PMID: 34437521] doi:10.15585/mmwr.mm7034e4
36. Reis BY, Barda N, Leshchinsky M, et al. Effectiveness of BNT162b2 vaccine against delta variant in adolescents [Letter]. N Engl J Med. 2021;385:2101-2103. [PMID: 34670036] doi:10.1056/NEJMc2114290
37. Li M, Lou F, Fan H. SARS-CoV-2 variants of concern Delta: a great challenge to prevention and control of COVID-19. Signal Transduct Target Ther. 2021;6:349. [PMID: 34580279] doi:10.1038/s41392-021-00767-1
38. Mallapaty S. China's COVID vaccines have been crucial – now immunity is waning. Nature. 2021;598:398-399. [PMID: 34650240] doi:10.1038/d41586-021-02796-w
39. Altmann DM, Boyton RJ. Waning immunity to SARS-CoV-2: implications for vaccine booster strategies. Lancet Respir Med. 2021;9:1356-1358. [PMID: 34688435] doi:10.1016/S2213-2600(21)00458-6

Author Contributions: Conception and design: J. He, M. Kang, Y. Li.
Analysis and interpretation of the data: Y. Jiang, M. Kang, Y. Yi.
Drafting of the article: Y. Jiang, Y. Yi.
Critical revision for important intellectual content: Y. Jiang, M. Kang, S. Tang.
Final approval of the article: M. Cheng, A. Deng, J. He, T. Hu, Y. Jiang, J. Jiang, M. Kang, Y. Li, J. Liu, M. Luo, L. Sun, S. Tang, S. Xie, Y. Yi, J. Zhang.
Statistical expertise: S. Tang.
Administrative, technical, or logistic support: J. He, Y. Li.
Collection and assembly of data: M. Cheng, A. Deng, T. Hu, J. Jiang, J. Liu, M. Luo, Y. Yi, J. Zhang.

Appendix: Appendix Methods

Close Contacts Management

Definition of Close Contacts

Close contacts are individuals who lived in the same household or stayed in the same public space without protection within close proximity in the 4 days before illness onset for symptomatic cases or sampling of the first positive specimen for asymptomatic cases. According to the epidemiologic investigation results and the digital information provided by government agencies, epidemiologic investigation professionals identify close contacts according to the following principles: 1) people who share living space; 2) direct caregivers or those who provide diagnosis, treatment, and nursing services; 3) health care workers who may be exposed to contaminated aerosols by conducting medical activities; 4) persons in close contact in the same place (for example, office, workshop, the same shift at workplace, elevator, canteen, or classroom); 5) persons who dine together, entertain together, and provide dining and entertainment services in a closed environment; 6) health care workers, family members, or other persons who attend an infected individual to provide medical or personal care; 7) persons who share a ride in the same vehicle and have close contact (within 1 meter) with an infected person, including caregivers and companions (for example, family members, colleagues, or friends); 8) persons exposed to environments and objects contaminated by infected persons; and 9) other persons who meet the criteria of close contact as assessed by onsite investigators.

Close Contacts Tracing

The local centers for disease control completed epidemiologic investigations of newly reported cases and traced and registered those patients' close contacts. They should have submitted the case investigation forms and close contact registration forms to the online reporting system as soon as possible. Close contact registration forms contain both sociodemographic and contact information, such as age, sex, address, occupation, times of last contact, and contact frequency.

Definition of Contact Frequency

"Occasionally" represented transient exposure, "sometimes" meant multiple nonenduring exposures, and "frequent" indicated multiple enduring exposures, such as people sharing a living space or workplace.

Management of Close Contacts

All close contacts were subject to quarantines following the 2-stage "14+7" model, which comprised a 14-day centralized quarantine stage and 1 week of home isolation (or centralized quarantine if self-isolation at home was not feasible).

For close contacts, the period of centralized quarantine for medical observation in designated facilities was 14 days after the last contact with a confirmed case patient or an asymptomatic infected person without effective protections.

The reverse transcriptase polymerase chain reaction tests were done on days 1, 4, 7, 10, and 14 during the centralized quarantine period for medical observation in designated facilities. Individuals released from quarantine practiced self-isolation at home for 7 days, during which they were tested again on days 2 and 7.

When the 21-day, 2-stage management period ended, the individual was dismissed from medical observation immediately if this person had no abnormal findings or symptoms.

Laboratory Confirmation

Four commercial reverse transcriptase polymerase chain reaction kits (DaAn Gene, BioGerm, BioPerfectus, and Easy Diagnosis) targeting the open reading frame (ORF1ab) and nucleocapsid protein genes were used to detect SARS-CoV-2 RNA during the outbreak.

When the case patients or close contacts were discharged from the hospital or released from quarantine, 2 nasopharyngeal swab samples should have been collected at the same time and tested with different reverse transcriptase polymerase chain reaction kits to avoid false negatives. In principle, the 2 tests are carried out by different testing institutions.

Adjusted Risk Ratio

After multivariable logistic regressions, the average risk for each outcome that would be expected if all participants in the analytic sample had received a specific vaccination exposure can be calculated. The ratio of the average predicted risks between 2 vaccination statuses represents the aRR of 1 group over the other. As such, the aRR was computed as the ratio of the average predicted risk (calculated over the entire sample) by setting the value of a specific exposure group indicator to 1 (that is, assuming everyone was in this group) over the average predicted risk by setting of the value of the reference exposure group indicator to 1 (assuming everyone was in the reference group) (29–31). For example, let P_{FV} be the mean of predicted probabilities of pneumonia over the entire analytic sample when vaccination status is

set to full vaccination, and let P_{NV} be the corresponding value when the vaccination status of everyone is set to no vaccination; then:

$$P_{FV} = \frac{1}{N}\sum_{i=1}^{N} \Pr(y_i = 1 | X,\ full\ vaccination = 1),$$

$$P_{FV} = \frac{1}{N}\sum_{i=1}^{N} \Pr(y_i = 1 | X,\ no\ vaccination = 1),$$

Adjusted RR is then calculated as P_{FV} / P_{NV}. When there are no covariates other than the vaccination status exposure variables, the RR estimated this way is the same as the unadjusted RR computed conventionally. The estimation can be implemented in Stata using the following example code:

```
logit pneumonia i.vaccination i.sex i.age_groups i.occupation i.subdistrict i.contact_frequency, or
margins i.vaccination, post
nlcom (aRR:_b[1.vaccination] / _b[0.vaccination])
```

In this example, "1.vaccination" stands for a specific vaccination status group–for example, the full vaccination group–whereas "0.vaccination" stands for the reference vaccination group–for example, the no-vaccination group.

Post Hoc Sensitivity Analyses

Post hoc sensitivity analyses were done to further examine the robustness of results, per reviewer recommendations. In these analyses, 1 change was made to the base case at a time. In the first post hoc sensitivity analysis, all vaccination status groups were included in the multivariable analyses of VE. Namely, the groups were the unvaccinated group, the intermediate first-dose group, the partially vaccinated group, the intermediate second-dose group, and the fully vaccinated group. In the second post hoc sensitivity analysis, clusters were taken into account in the multivariable logistic regressions. The close contacts of each case in the transmission chain made up a cluster. Clusters could potentially affect the estimates of SEs of VEs. To that end, we estimated the VEs by using multivariable logistic regressions with cluster-robust SEs in this sensitivity analysis. In the third post hoc sensitivity analysis, the multivariable analyses in the base case were repeated using Poisson regressions in lieu of logistic regressions. The outputs from the multivariable Poisson regressions were incidence rate ratios. In all post hoc sensitivity analyses, the specification of covariates in the multivariable regressions remained the same as that in the base-case analysis.

E-Value

To test the robustness of the VE estimates to potential unmeasured confounders, we computed the E-values for both the aRRs and the upper bounds of their CIs (34). In observational studies, estimates of causal effects may be biased by confounders that correlate with both the exposure of interest (for example, fully vaccinated) and the outcomes (for example, pneumonia). When a confounder has a sufficiently sizeable amount of correlation with both the exposure and the outcome, the estimated effects in observational studies may be nullified. That is, the observed effects may be fully attributable to confounding bias rather than true effects. The E-value is a single metric that quantifies the sufficiently sizeable amount of correlation that an unmeasured confounder would need to have with both a specific vaccination status and a certain outcome to negate the observed VE (in the scale of RR) after adjustment for the measured covariates. As such, a larger E-value suggests stronger required confounder associations with the exposure and the outcome to explain away the observed VE. Of note, the aRRs should be less than 1 for VEs to exist; the upper bounds of statistically insignificant aRRs were greater than 1 to begin with. By definition, the E-values of such upper bounds were 1 (34).

Based on the results in **Appendix Table 7**, the E-values for full vaccination ranged from 3.6 to 8.7 and the range of E-values for the upper bounds of CIs was 1.8 to 4.1 for the outcomes of infection, symptomatic infection, and pneumonia, indicating that moderate to strong confounder associations with full vaccination and the outcomes needed to be present simultaneously to explain away the observed VE. Specifically, the statistical significance of the VE of full vaccination for infection could be explained away if there existed an unmeasured confounder that was associated with both full vaccination and infection with a strength at least as large as an RR of 1.8. The corresponding E-values of the VE of full vaccination for symptomatic infection and pneumonia were 2.3 and 4.1, respectively.

Estimation of CIs for Groups With Zero-Event Cells

The estimation of CIs for groups with zero-event cells was based on Bayesian binomial regressions as proposed by Möller and Ahrenfeldt (40). A set of example code to conduct this analysis in Stata is provided below.

```
bayes, nomleinitial noi rseed(20211015): binreg severe i.vaccination, rr asis
```

Web Reference

40. Möller S, Ahrenfeldt LJ. Estimating relative risk when observing zero events–frequentist inference and Bayesian credibility intervals. Int J Environ Res Public Health. 2021;18. [PMID: 34064019] doi:10.3390/ijerph18115527

Appendix Table 1. Number of Individuals, by Vaccination Status, Across Vaccine Brands*

Brand	Unvaccinated (*n* = 5888 [54.5%])	Intermediate 1st Dose (*n* = 2286 [21.1%])	Partially Vaccinated (*n* = 841 [7.8%])	Intermediate 2nd Dose (*n* = 387 [3.6%])†	Fully Vaccinated (*n* = 1403 [13.0%])‡	Total (*n* = 10 805)
First dose						
HB02	NA	1316 (57.6)	333 (39.6)	146 (37.7)	597 (42.6)	2392 (48.6)
CoronaVac	NA	970 (42.4)	508 (60.4)	241 (62.3)	806 (57.4)	2525 (51.4)
Second dose						
HB02	NA	NA	NA	163 (42.1)	581 (41.4)	744 (41.6)
CoronaVac	NA	NA	NA	224 (57.9)	822 (58.6)	1046 (58.4)

NA = not applicable.
* Values are numbers (percentages).
† 105 people received a different brand for the 2nd dose.
‡ 268 people received a different brand for the 2nd dose.

Appendix Table 2. aRRs From Multivariable Logistic Regressions*

Covariate	Infection	Symptomatic Infection	Pneumonia
Vaccination status (reference: unvaccinated)			
Partially vaccinated	0.893 (0.374 to 1.412)	0.932 (0.39 to 1.474)	0.884 (0.342 to 1.426)
Fully vaccinated	0.482 (0.168 to 0.797)	0.396 (0.111 to 0.682)	0.216 (0.001 to 0.431)
Sex (reference: female)	0.637 (0.41 to 0.865)	0.635 (0.406 to 0.864)	0.521 (0.318 to 0.724)
Age group (reference: 18-34 y)			
35-49 y	1.566 (0.603 to 2.529)	1.409 (0.524 to 2.294)	1.844 (0.489 to 3.199)
≥50 y	2.711 (1.215 to 4.207)	2.668 (1.193 to 4.143)	3.683 (1.263 to 6.102)
Occupation (reference: other)			
Restaurant services	3.786 (0.809 to 6.763)	3.218 (0.387 to 6.048)	2.966 (−0.022 to 5.954)
Unemployed/home	7.465 (4.373 to 10.557)	7.251 (4.2 to 10.302)	7.357 (4.197 to 10.517)
Health care worker	3.356 (−0.237 to 6.949)	3.599 (−0.24 to 7.438)	3.762 (−0.962 to 8.485)
Subdistrict (reference: other)			
A	5.076 (1.795 to 8.356)	4.76 (1.574 to 7.946)	5.918 (1.893 to 9.943)
B	8.145 (5.038 to 11.251)	8.345 (5.125 to 11.565)	8.893 (5.236 to 12.55)
Contact frequency (reference: sometimes)			
Occasionally	1.53 (0.883 to 2.176)	1.476 (0.838 to 2.114)	1.528 (0.827 to 2.23)
Frequently	13.607 (8.114 to 19.099)	14.046 (8.373 to 19.719)	15.669 (9.115 to 22.223)

aRR = adjusted risk ratio.
* Values are aRRs (95% CIs).

Appendix Table 3. aVE of Preventing Infections, Symptomatic Infections, and Pneumonia, by Vaccination Status Defined Using Number of Doses Before 7 May 2021 (14 Days Before First Report of the Outbreak)

Outcome and Vaccination Status	Events/Participants, *n/N* (% [95% CI])	Unadjusted VE (95% CI), %	aVE (95% CI), %*
Infection			
Unvaccinated	92/4678 (2.0 [1.6 to 2.4])	Reference	-
Partially vaccinated	22/1475 (1.5 [0.9 to 2.2])	24.2 (−10.8 to 59.2)	22.5 (−13.6 to 58.5)
Fully vaccinated	8/1049 (0.8 [0.3 to 1.5])	61.2 (33.3 to 89.1)	58.1 (27.7 to 88.5)
Symptomatic infection			
Unvaccinated	91/4678 (1.9 [1.6 to 2.4])	Reference	-
Partially vaccinated	22/1475 (1.5 [0.9 to 2.2])	23.3 (−12.1 to 58.8)	19.5 (−17.9 to 57.0)
Fully vaccinated	6/1049 (0.6 [0.2 to 1.2])	70.6 (46.4 to 94.8)	67.7 (41.0 to 94.5)
Pneumonia			
Unvaccinated	84/4678 (1.8 [1.4 to 2.2])	Reference	-
Partially vaccinated	18/1475 (1.2 [0.7 to 1.9])	32.0 (−2.3 to 66.4)	29.3 (−6.7 to 65.4)
Fully vaccinated	4/1049 (0.4 [0.1 to 1.0])	78.8 (57.5 to 100.0)	76.5 (52.8 to 100.3)

aVE = adjusted vaccine effectiveness; VE = vaccine effectiveness.
* Adjusted for sex, age, occupation, subdistrict, and contact frequency.

Appendix Table 4. VE in Preventing Infections, Symptomatic Infections, and Pneumonia, by the 5-Category Vaccination Status

Outcome and Vaccination Status	Events/Participants, *n/N (% [95% CI])*	aRR (95% CI)*	aVE (95% CI), %*
Infection			
Unvaccinated	93/5888 (1.6 [1.3 to 1.9])	Reference	-
Intermediate 1st dose	16/2286 (0.7 [0.4 to 1.1])	0.744 (0.369 to 1.118)	25.6 (−11.8 to 63.1)
Partially vaccinated	13/841 (1.5 [0.8 to 2.6])	0.857 (0.356 to 1.357)	14.3 (−35.7 to 64.4)
Intermediate 2nd dose	4/387 (1.0 [0.3 to 2.6])	0.848 (0.062 to 1.634)	15.2 (−63.4 to 93.8)
Fully vaccinated	10/1403 (0.7 [0.3 to 1.3])	0.477 (0.170 to 0.784)	52.3 (21.6 to 83.0)
Symptomatic infection			
Unvaccinated	92/5888 (1.6 [1.3 to 1.9])	Reference	-
Intermediate 1st dose	16/2286 (0.7 [0.4 to 1.1])	0.773 (0.384 to 1.162)	22.7 (−16.2 to 61.6)
Partially vaccinated	13/841 (1.5 [0.8 to 2.6])	0.888 (0.369 to 1.408)	11.2 (−40.8 to 63.1)
Intermediate 2nd dose	4/387 (1.0 [0.3 to 2.6])	0.860 (0.063 to 1.657)	14.0 (−65.7 to 93.7)
Fully vaccinated	8/1403 (0.6 [0.2 to 1.1])	0.391 (0.113 to 0.670)	60.9 (33.0 to 88.7)
Pneumonia			
Unvaccinated	85/5888 (1.4 [1.2 to 1.8])	Reference	-
Intermediate 1st dose	16/2286 (0.7 [0.4 to 1.1])	0.881 (0.439 to 1.322)	11.9 (−32.2 to 56.1)
Partially vaccinated	12/841 (1.4 [0.7 to 2.5])	0.836 (0.321 to 1.351)	16.4 (−35.1 to 67.9)
Intermediate 2nd dose	3/387 (0.8 [0.2 to 2.2])	0.741 (−0.040 to 1.522)	25.9 (−52.2 to 104.0)
Fully vaccinated	4/1403 (0.3 [0.1 to 0.7])	0.211 (0.002 to 0.419)	78.9 (58.1 to 99.8)

aRR = adjusted risk ratio; aVE = adjusted vaccine effectiveness; VE = vaccine effectiveness.
* Adjusted for sex, age, occupation, subdistrict, and contact frequency.

Appendix Table 5. VE in Preventing Infections, Symptomatic Infections, and Pneumonia, by Vaccination Status, Using Cluster-Robust SEs

Outcome and Vaccination Status	Events/Participants, *n/N (% [95% CI])*	aRR (95% CI)*	aVE (95% CI), %*
Infection			
Unvaccinated	93/5888 (1.6 [1.3 to 1.9])	Reference	-
Partially vaccinated	13/841 (1.5 [0.8 to 2.6])	0.893 (0.384 to 1.402)	10.7 (−40.2 to 61.6)
Fully vaccinated	10/1403 (0.7 [0.3 to 1.3])	0.482 (0.138 to 0.826)	51.8 (17.4 to 86.2)
Symptomatic infection			
Unvaccinated	92/5888 (1.6 [1.3 to 1.9])	Reference	-
Partially vaccinated	13/841 (1.5 [0.8 to 2.6])	0.932 (0.404 to 1.460)	6.8 (−46.0 to 59.6)
Fully vaccinated	8/1403 (0.6 [0.2 to 1.1])	0.396 (0.099 to 0.694)	60.4 (30.6 to 90.1)
Pneumonia			
Unvaccinated	85/5888 (1.4 [1.2 to 1.8])	Reference	-
Partially vaccinated	12/841 (1.4 [0.7 to 2.5])	0.884 (0.381 to 1.386)	11.6 (−38.6 to 61.9)
Fully vaccinated	4/1403 (0.3 [0.1 to 0.7])	0.216 (0.039 to 0.393)	78.4 (60.7 to 96.1)

aRR = adjusted risk ratio; aVE = adjusted vaccine effectiveness; VE = vaccine effectiveness.
* Adjusted for sex, age, occupation, subdistrict, contact frequency, and cluster.

Appendix Table 6. VE in Preventing Infections, Symptomatic Infections, and Pneumonia, by Vaccination Status, Using Poisson Regressions

Outcome and Vaccination Status	IRR (95% CI)*	aVE (95% CI), %*†
Infection		
Unvaccinated	Reference	-
Partially vaccinated	0.950 (0.514 to 1.755)	5.0 (-75.5 to 48.6)
Fully vaccinated	0.478 (0.237 to 0.964)	52.2 (3.6 to 76.3)
Symptomatic infection		
Unvaccinated	Reference	-
Partially vaccinated	0.989 (0.534 to 1.831)	1.1 (-83.1 to 46.6)
Fully vaccinated	0.394 (0.182 to 0.852)	60.6 (14.8 to 81.8)
Pneumonia		
Unvaccinated	Reference	-
Partially vaccinated	0.946 (0.495 to 1.808)	5.4 (-80.8 to 50.5)
Fully vaccinated	0.211 (0.074 to 0.603)	78.9 (39.7 to 92.6)

aVE = adjusted vaccine effectiveness; IRR = incidence rate ratio; VE = vaccine effectiveness.
* Adjusted for sex, age, occupation, subdistrict, and contact frequency.
† Calculated as 1 - IRR.

Appendix Table 7. E-Values for aRRs of Vaccination Statuses From the Base-Case Multivariable Logistic Regressions*

Covariate	Infection	Symptomatic Infection	Pneumonia
Vaccination status (reference: unvaccinated)			
Partially vaccinated	1.5 (1.0)	1.4 (1.0)	1.5 (1.0)
Fully vaccinated	3.6 (1.8)	4.5 (2.3)	8.7 (4.1)

aRR = adjusted risk ratio.
* Results are E-values for the aRR (E-values for the upper bound of the CI of aRR).

2.4. Três doses da CoronaVac induzem anticorpos contra a ômicron em 95% dos vacinados, mostra estudo chinês

Em um trabalho publicado na revista Nature, pesquisadores da Academia Chinesa de Ciências mostraram que a dose de reforço da CoronaVac promove resposta imune contra a variante ômicron do SARS-CoV-2 em 95% dos vacinados, além de aumentar a capacidade de neutralização dessa cepa ao ativar rapidamente as células B de memória, que produzem anticorpos.

Os cientistas chineses coletaram amostras de sangue de 60 voluntários que tomaram três doses da CoronaVac para avaliar os títulos de anticorpos neutralizantes contra as variantes ômicron e delta – neste estudo, usou-se vírus vivo. Nenhum dos indivíduos recrutados tinha sido infectado pelo vírus SARS-CoV-2 antes da análise.

Segundo a pesquisa, após a terceira dose, 95% dos participantes apresentaram soroconversão contra a ômicron. Os títulos de anticorpos neutralizantes contra a cepa original (de Wuhan, que desencadeou a pandemia) e contra as variantes delta e ômicron foram, respectivamente, 254, 78 e 15,5. A contagem de títulos de anticorpos, no entanto, representa apenas uma parte da resposta imune, que é completada pelas células B de memória, que podem reconhecer um invasor, se dividir e rapidamente começar a produzir anticorpos para combatê-lo.

Para avaliar o potencial da memória imunológica de vacinados com três doses, os cientistas isolaram 323 anticorpos monoclonais derivados de células B, metade dos quais (163) reconheceu o domínio de ligação ao receptor (RBD) do vírus. Também foi identificado um subconjunto dos anticorpos monoclonais (24 dos 163) que foi capaz de neutralizar todas as variantes de preocupação do SARS-CoV-2, inclusive a ômicron.

De acordo com os pesquisadores, estudos têm mostrado que a ômicron pode resistir aos anticorpos produzidos com duas doses de vacina, o que reforça a necessidade de uma terceira dose. "Nosso estudo revelou que o regime de vacinação de três doses da CoronaVac induz uma resposta imune melhorada, com neutralização significativamente aumentada. Além disso, um subconjunto de anticorpos neutralizantes altamente potentes contra as variantes de preocupação estava presente em pelo menos quatro indivíduos [entre 60 investigados]."

Publicado em: 28/1/2022

nature

https://doi.org/10.1038/s41586-022-04466-x

Accelerated Article Preview

Memory B cell repertoire from triple vaccinees against diverse SARS-CoV-2 variants

Received: 22 December 2021

Accepted: 20 January 2022

Accelerated Article Preview
Published online: 28 January 2022

Cite this article as: Wang, K. . et al. Memory B cell repertoire from triple vaccinees against diverse SARS-CoV-2 variants. *Nature* https://doi.org/10.1038/s41586-022-04466-x (2022).

Kang Wang, Zijing Jia, Linlin Bao, Lei Wang, Lei Cao, Hang Chi, Yaling Hu, Qianqian Li, Yinan Jiang, Qianhui Zhu, Yongqiang Deng, Pan Liu, Nan Wang, Lin Wang, Min Liu, Yurong Li, Boling Zhu, Kaiyue Fan, Wangjun Fu, Peng Yang, Xinran Pei, Zhen Cui, Lili Qin, Pingju Ge, Jiajing Wu, Shuo Liu, Yiding Chen, Weijin Huang, Cheng-Feng Qin, Youchun Wang, Chuan Qin & Xiangxi Wang

Artigo

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

Memory B cell repertoire from triple vaccinees against diverse SARS-CoV-2 variants

Kang Wang,[1,8] Zijing Jia,[1,8] Linlin Bao,[2,8] Lei Wang,[1,7,8] Lei Cao,[1,8] Hang Chi,[3,8] Yaling Hu,[4,8] Qianqian Li,[5,8] Yinan Jiang,[6] Qianhui Zhu,[1,7] Yongqiang Deng,[3] Pan Liu,[1] Nan Wang,[1] Lin Wang,[4] Min Liu,[4] Yurong Li,[4] Boling Zhu,[1] Kaiyue Fan,[1,7] Wangjun Fu,[1,7] Peng Yang,[1,7] Xinran Pei,[1] Zhen Cui,[1,7] Lili Qin,[6] Pingju Ge,[6] Jiajing Wu,[5] Shuo Liu,[5] Yiding Chen,[6] Weijin Huang,[5] Cheng-Feng Qin,[3]† Youchun Wang,[5]† Chuan Qin,[2]† & Xiangxi Wang,[1,7]†

[1]CAS Key Laboratory of Infection and Immunity, National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China.

[2]Key Laboratory of Human Disease Comparative Medicine, Chinese Ministry of Health, Beijing Key Laboratory for Animal Models of Emerging and Remerging Infectious Diseases, Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Comparative Medicine Center, Peking Union Medical College, Beijing, China.

[3]State Key Laboratory of Pathogen and Biosecurity, Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, China.

[4]Sinovac Biotech Ltd, Beijing, China.

[5]Division of HIV/AIDS and Sex-Transmitted Virus Vaccines, Institute for Biological Product Control, National Institutes for Food and Drug Control (NIFDC), Beijing 102629, China.

[6]Acrobiosystems Inc, Beijing, China.

[7]University of Chinese Academy of Sciences, Beijing 100049, China.

[8]These authors contributed equally: Kang Wang, Zijing Jia, Linlin Bao, Lei Wang, Lei Cao, Hang Chi, Yaling Hu, Qianqian Li.

†**e-mail:** qincf@bmi.ac.cn; wangyc@nifdc.org.cn; qinchuan@pumc.edu.cn; xiangxi@ibp.ac.cn

Omicron, the most heavily mutated SARS-CoV-2 variant so far, is highly resistant to neutralizing antibodies, raising unprecedented concerns about the effectiveness of antibody therapies and vaccines [1,2]. We examined whether sera from individuals who received two or three doses of inactivated vaccine, could neutralize authentic Omicron. The seroconversion rates of neutralizing antibodies were 3.3% (2/60) and 95% (57/60) for 2- and 3-dose vaccinees, respectively. For three-dose recipients, the geometric mean neutralization antibody titre (GMT) of Omicron was 16.5-fold lower than that of the ancestral virus (254). We isolated 323 human monoclonal antibodies (mAbs) derived from memory B cells in 3-dose vaccinees, half of which recognize the receptor binding

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

domain (RBD) and show that a subset of them (24/163) neutralize all SARS-CoV-2 variants of concern (VOCs), including Omicron, potently. Therapeutic treatments with representative broadly neutralizing mAbs were highly protective against SARS-CoV-2 Beta and Omicron infections in mice. Atomic structures of the Omicron Spike in complex with three types of all five VOC-reactive antibodies defined the binding and neutralizing determinants and revealed a key antibody escape site, G446S, that confers greater resistance to one major class of antibodies bound at the right shoulder of RBD through altering local conformation at the binding interface. Our results rationalize the use of 3-dose immunization regimens and suggest that the fundamental epitopes revealed by these broadly ultrapotent antibodies are a rational target for a universal sarbecovirus vaccine.

The ongoing evolution and emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants raise concerns about the effectiveness of monoclonal antibodies (mAbs) therapies and vaccines [3-5], posing challenges for global pandemic control. These variants were characterized as Variant of Interest, VOI or Variant of Concern, VOC by the World Health Organization (WHO). The more recently identified Omicron variant (B.1.1.529), designated as a new VOC, has led to an unprecedented surge in COVID-19 cases in South Africa and is now spreading across the world [6]. Remarkably, Omicron is the most heavily mutated variant to emerge so far with over thirty mutations in spike (S) protein, fifteen of which occur in the receptor binding domain (RBD). In addition, there are three small deletions and one 3-residue insertion in the N-terminal domain (NTD) of S1 subunit (Fig. 1a). The pattern of some of these alterations, similar to the those noted in previous VOCs, such as Δ69-70 in Alpha, N501Y in Alpha, Beta and Gamma, P681H in Alpha and Delta, are presumably associated with enhanced transmissibility, while many substitutions, including G142D/Δ143-145, ins214EPE, K417N, T478K, E484A, Q493R and N501Y, are closely related with resistance to neutralizing antibodies and vaccine induced humoral immunity [3,5,7-11] (Fig. 1a and 1b).

Although COVID-19 vaccines continued to be effective against severe diseases and deaths, including those caused by the circulating Delta variant, waning immunity and massive breakthrough infections caused by viral diversification warrant the need for a third dose or new vaccines. To combat the current resurgence of the

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

epidemic, the U.S. Food and Drug Administration has authorized use of a 3rd booster dose for all adults after completion of primary vaccination with approved COVID-19 vaccine [12]. This step seems essential because preliminary studies have indicated that three doses of Pfizer-BioNtech mRNA vaccine neutralize the Omicron variant with an approximate 40-fold decline, while two doses are less effective [1,13]. However, these preliminary data on the neutralization sensitivity of Omicron require further independent confirmation. The clinical impact of natural and vaccine-induced immunity with regards to protection from infection and severe disease needs urgent investigation.

Authentic Omicron neutralization

The CoronaVac, a β-propiolactone-inactivated vaccine against COVID-19, has been approved for emergency use, and recommended for a booster dose (third) of inactivated vaccine in older persons by WHO [14,15]. Serum specimens from two groups of 2-dose (n=60, at month 0, 1) or 3-dose (n=60, at months 0, 1, 7) CoronaVac vaccinee volunteers were collected for evaluating neutralization titers against the Omicron and Delta variants using a live SARS-CoV-2. None of the volunteers recruited for vaccination was infected by SARS-CoV-2 prior to the study. Blood samples from vaccinees collected 4 weeks after the last vaccination were used in this study, to compare NAb titers against circulating SARS-CoV-2 variants. An early passage of isolated (CHK06 strain) and sequence confirmed live Omicron virus was used for neutralization assay in this study. Among three doses of CoronaVac recipients, the geometric mean half-maximal neutralizing titers (GMT NT_{50}) against live wild-type (WT) virus, Delta and Omicron variants were 253.9, 77.8 and 15.4, respectively. Compared with WT, neutralizing titers against Delta and Omicron were, on average, 3.3-fold and 16.5-fold reduced, respectively (Fig. 1c). Only 3 of 60 samples had a NT_{50} titer of < 8 against the Omicron with a seroconversion rate of 95% for neutralizing antibodies (Fig. 1c). However, it's more concerning about effectiveness for two-dose regime against Omicron infection. Among two doses of CoronaVac recipients, NT_{50} titer against Delta was 6.6 with a 5.1-fold reduction when compared to WT, but none of the serum specimens had an NT_{50} titer of >8 against Omicron (Fig. 1c). Compared to 2-dose vaccinees, sera of the 3-dose vaccinees displayed lower reduction in neutralization titers against Delta, which is consistent

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

with previous observations that 3-dose administration of inactivated vaccine leads to enhanced neutralizing breadth to SARS-CoV-2 variants [7].

MAbs elicited by 3-dose vaccination

We previously sorted immunoglobulin (IgG+) memory B cells from peripheral blood mononuclear cells (PBMCs) of four 3-dose CoronaVac vaccinees using prefusion SARS-CoV-2 S as a bait [7,16]. In total, we sorted 1,800 SARS-CoV-2 S-specific memory B cells, obtained 422 paired heavy- and light-chain antibody sequences, and selected 323 antibodies for expression (Supplementary Table 1). Characterization by ELISA showed that 163, 100 and 51 recognized the RBD, NTD and S2, respectively and 9 failed to bind S (Fig. 2a). Biolayer interferometry affinities (BLI) measurements showed that nearly all RBD-directed antibodies bound to WT SARS-CoV-2 at sub-nM levels (Supplementary Table 1) and 127 of them showed neutralization activities against both authentic and pseudotyped WT SARS-CoV-2 were selected for further investigation. Of these antibodies, over 93% of these antibodies exhibited broad binding activities to most VOCs and VOIs (Supplementary Table 1). Notably, 85% of these antibodies cross-reacted with the Omicron RBD (Supplementary Table 1). Contrarily, ~80% of NTD antibodies lost their associations with Omicron. Additionally, NTD antibodies also showed relatively poor cross-reactivity to other four VOCs due to the greater diversity of the NTD (Fig. 1a, b and Supplementary Table 1).

MAbs with broad neutralization

Results of the pseudovirus neutralization assays performed by carrying the S of WT or other VOCs [17,18] identified 31 RBD targeting antibodies that were especially potent with their half-maximal inhibitory concentration (IC_{50}) ranging from 0.002 to 0.800 μg/ml against WT as well as all VOCs (Fig. 2b). Among these, 30 antibodies executed their neutralization via directly blocking the interactions between the RBD and its receptor hACE2, while 1 antibody employs other mechanisms to neutralize viral infection (Fig. 2c, Extended Data Fig. 1). Especially, a subset of RBD antibodies (13 and 24) neutralized Omicron with $IC_{50} < 0.02$ and 0.1 μg/ml, respectively. These neutralizations are as potent as those exhibited by best-in-class antibodies against WT (Fig. 2b and 2d, Supplementary Table 1, 2). We obtained IC_{50} values of 0.27 and 0.16 μg/ml for well-studied therapeutic antibodies like VIR-7831 and DXP-604,

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

respectively. These values are 10~40-fold higher than those of the subset antibodies (Extended Data Fig. 2, Supplementary Table 1). Concerningly, some antibody drugs, such as REGN10933, REGN10987, LY-CoV555, LY-CoV016, AZD1061 and AZD8895, almost lost their neutralization activities against Omicron (Extended Data Fig. 2, Supplementary Table 1)[2]. Meanwhile, specific VOC-resistant antibodies with high neutralizing potency against WT and some other VOCs (IC_{50} <0.2 μg/ml) were identified and these comprise ~30% of the antibody repertoire (Supplementary Data Table 1). Our previous study revealed that the numbers of nucleotide mutations in the V gene for RBD specific antibodies in 3-dose vaccinees were substantially higher than those in 2-dose vaccinees and antibodies obtained from 3-dose vaccinees possessed higher binding activities than those from 2-dose vaccinated individuals[5], which indicates the evolution of a wide range of antibodies over time. Experiments repeated using authentic virus, including WT and five circulating VOCs, showed similar neutralization patterns by all these antibodies (Extended Data Fig. 3), further verifying the neutralizing potency and breadth for this subset of antibody repertoire elicited by 3-dose vaccination.

Structures of Omicron S trimer and mAbs

Antibodies targeting the RBD can be categorized into six general classes (from I to VI) based on cluster analysis on epitope from 265 available RBD-NAb complex structures [7], that are related to the four groups on the basis of competition with the hACE2 for binding to S and recognition of the up or down state of the three RBDs in S [19-21]. ELISA-based square competition matrix analysis with the aid of existing structural data revealed the presence of 3 major groups in this subset of antibody repertoire (Extended Data Fig. 4). To delineate the structural basis for antibody-mediated neutralization, we determined the cryo-EM structure of a prefusion stabilized Omicron S trimer in complex with representative Fab fragments. The two highly potent antibodies against Omicron (XGv347 and XGv289 with IC_{50} values of 0.006 and 0.016 μg/ml, respectively), one mAb (XGv282 with IC_{50} of 0.268 μg/ml) with median neutralizing activities against Omicron, but high neutralizing potency against other four VOCs, and one mAb (XGv265 with IC_{50} of 7.479 μg/ml) with >500-fold decreased neutralization against Omicron, but potent neutralization against other four VOCs were selected for structural investigations (Fig. 2b). We determined cryo-EM reconstructions of these complexes at 3.3 – 3.8 Å, and performed local

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

refinement to further improve the densities around the binding interface between RBD and antibodies, enabling reliable analysis of the interaction details (Fig. 3, Extended Data Fig. 5, 6 and 7, Extended Data Table 1).

The XGv347-Omicron S complex structures revealed three distinct conformational states: three XGv347 Fabs bound to a completely closed S with three down RBDs; two XGv347 Fabs bound to either two or one up and one down RBDs on S (Fig. 3a). By contrast, each of the complex structures for XGv289, XGv282 and XGv265 showed only one configuration where three XGv289 Fabs bound to two up and one down RBDs; three XGv282 Fabs bound to one up and two down RBDs; two XGv265 Fabs bound to S trimer with one down and one up RBD, although the XGv265-bound up RBD conformation was weakly resolved and therefore not modeled (Fig. 3a). Antibody XGv347 binds to an epitope at the tip of RBD, largely overlapping with the patch targeted by ACE2 (Fig. 2c, 3b, 3c, Extended Data Fig. 1). Structural comparisons revealed that XGv347 is very similar to A23-58.1, an ultrapotent and broadly reactive NAb effective against 23 SARS-CoV-2 variants [22], but significant differences could be observed in the CDR domains (Extended Data Fig. 8). Furthermore, the residues of the epitope of XGv347 match with a major subset of those targeted by S2K146, another broadly cross-reactive sarbecovirus NAb [23,24], highlighting a plausible capability of these NAbs to cross-neutralize Omicron and circulating SARS-CoV-2 variants. Unexpectedly, the epitopes of XGv347, A23-58.1 as well as their sister NAbs would be normally inaccessible for the RBD-down conformation in the WT S, but become accessible for either up or down RBDs in the Omicron S due to a markedly outward expansion and a ~10° clockwise rotation of three RBDs, leading to an approximately 9 Å conformational movement for RBM (Fig. 3d and Extended Data Fig. 9). The XGv347 paratope constituted five complementarity determining regions (CDRs) with heavy chain and light chain contributing 70% and 30% of the binding surface area, respectively (Fig. 3b, 3c and Extended Data Table 2). Overall XGv289, XGv282 and XGv265 bind patches surrounding the right shoulder of RBD with various orientations [20], but in a manner similar to those observed for DH1047, BD-812 and REGN10987; antibodies known to generally neutralize most VOCs with high potency [25-27], but showing declined, to varying degrees, binding and neutralizing activities against Omicron due to the presence of new N440K and G446S mutations (Fig. 2b, Extended Data Fig. 10 and

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

Extended Data Table 2). Notably, XGv265 and REGN10987 recognize almost same epitopes, both nearly losing their neutralizing activities against Omicron, despite retaining weak binding (Extended Data Fig. 10). Structural superimpositions and competitive BLI assays reveal that XGv347 and either XGv289 or XGv265 can simultaneously bind to S, informing strategies to rationally design two-antibody combinations for potential therapeutics (Extended Data Fig. 11, Extended Data Fig. 12).

Structural basis for immune escape

XGv347, XGv289, XGv282 and XGv265 bound Omicron with 5-40 folds lower affinity compared to their binding with WT, although the same binding modes for two orthologs were observed (Fig. 3 and Supplementary Table 1). For XGv347, tight binding to WT S is primarily due to extensive hydrophobic interactions contributed by F456, Y473, F486 and Y489 from WT RBD, V32, V53, W51, P100 and F111 from heavy chain, and Y33 from light chain, and 9 hydrogen bonds (Extended Data Fig. 13 and Table 3). Hydrophobic interactions between the Omicron RBD and XGv347 are largely maintained. However, substitutions of Y505H and K417N abolish three hydrogen bonds forged with K75, D31 and E104 from HCDRs, leading to conformational shifts in HCDR3 and the RBM tip (residues 470-490), which further perturb six hydrogen bonds built by Y473, A475, S477, T478, Q493 from WT RBD with T105, C107, A56, G55 and D109 from HCDRs, albeit with an extra hydrogen bond established by the mutation Q493R and G55 from HCDR2 in Omicron (Extended Data Fig. 13). Similarly, a large patch of hydrophobic interactions constructed by V445, G446, Y449, P499 from WT RBD and F33, L50, I51, Y59, W103 from HCDRs as well as extensive hydrophilic interactions facilitate tight binding between XGv289 and WT S (Fig. 3 and Extended Data Fig. 13). Substitution of G446S disrupts the hydrophobic microenvironment, substantially decreasing hydrophobic interactions between Omicron S and XGv289. Furthermore, mutations of N440K and Q498R, together with altered local conformation, also lessen hydrogen bonds formed by N439, K440, Y449, R498, T500, Q506 from Omicron RBD and D95, L98 from LCDRs as well as Y59, N62 from HCDRs that would exist in XGv289-WT S complex (Extended Data Fig. 13). Among these four representative antibodies, XGv282 showed minimal reduction in binding affinity (5-fold), but remarkable reduction in neutralization (~40-fold), versus the characterization of

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

XGv347 with 40-fold decrease in binding, but unchanged neutralization against Omicron when compared to WT (Extended Data Table 3), suggesting that epitope, rather than binding affinity, might play more crucial roles in the neutralizing potency and breadth of an antibody. Consistent with XGv289, the substitution of G446S alters the hydrophobic microenvironment generally established by RBD and a group of antibodies bound at the right shoulder, including XGv289 and XGv282, triggering a conformational shift on CDRs and disrupting antibody recognition (Extended Data Fig. 13). In addition, the mutation E484A breaks hydrogen bond-connection with R74 from XGv282 HCDR2 and losses of charge interactions between R346, K444 from WT RBD and D56, D58 of XGv265 LCDR2 due to conformational alterations, further decreasing the binding of XGv282 and XGv265 to the Omicron variant, respectively (Extended Data Fig. 13). Taken together, G446S, acting as a critical mutation site, can alter the local conformation at the binding interface, conferring greater resistance to one class of antibodies bound at the right shoulder of RBD.

The therapeutic activities of mAbs

Given the excellent neutralizing breadth and potency at cell-based levels for above antibodies, we next sought to assess the correlation between *in vitro* neutralization and *in vivo* protection. A number of representative mAbs with high neutralizing potency and breadth, belonging to different classes, such as XGv347, XGv289, XGv282, XGv265 and XGv052, produced in the HEK293F cell line were selected for therapeutic evaluation in a well-established mouse model challenged with the Beta variant [28]. Upon Beta intranasal challenge, adult BALB/c showed robust viral replication in the lungs at 3-5 days post inoculation (dpi). To evaluate the protection efficacy of these mAb, BALB/c mice challenged with the Beta variant were administered a single dose of as low as 5 mg/kg of XGv347, XGv289, XGv282, XGv265 and XGv052 individually or combinations of XGv282 and XGv347 (2.5 mg/kg for each), and XGv052 and XGv289 (2.5 mg/kg for each) in therapeutic settings (Fig. 4a). Heavy viral loads with high levels of viral RNAs (> 10^9 copies/g) were detected in the lungs at day 5 post-infection in the control group of mice treated with PBS. However, a single dose of XGv282 reduced the viral RNA loads by ~10,000-fold in the lungs compared to the control group (Fig. 4b). Remarkably, a single dose of XGv289, XGv265, XGv347, XGv052 or antibody cocktails of XGv282 and XGv347, XGv052 and XGv289 resulted in a complete clearance of viral particles

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

in the lungs (Fig. 4b, 4c). A potential synergistic effect was observed for combined therapies of XGv282 + XGv347 at 2.5 mg/kg for each (Fig. 4b, 4c). In addition, histopathological examination revealed severe interstitial pneumonia, characterized by alveolar septal thickening, inflammatory cell infiltration and distinctive vascular system injury developed in mice belonging to the control group at day 5 (Fig. 4d). In contrast, no obvious lesions of alveolar epithelial cells or focal hemorrhage were observed in the lung sections from mice that received indicated antibody treatments (Fig. 4d, Extended Data Fig. 14). To further evaluate whether XGv347 could serve as therapeutic interventions against Omicron *in vivo*, we tested the protective efficacy of XGv347 on hACE2 transgenic mice challenged by Omicron. We recorded the body weight for each mouse daily after infection for 5 days and found that the therapeutic treatment group maintained their body weight, whereas the control group substantially lost weight (Fig. 4e), indicating that XGv347 applied after the infection could greatly improve the physiological condition of the Omicron-infected mice. Similar to the studies with the Beta strain of mice, therapeutic administration of XGv347 conferred a clear benefit on the hACE2 transgenic mouse model (K18-hACE2) [29] as indicated by a complete clearance in viral RNA loads in the lungs and trachea at day 5 post Omicron challenge (Fig. 4f). More importantly, K18-hACE2 mice infected with Omicron developed moderate interstitial pneumonia characterized by focal to multifocal widen alveolar interstitium accompanied by infiltration of inflammatory cells (Fig. 4g). While, no obvious pathological injury was observed in the lung from mice that received XGv347 treatments (Fig. 4g). Collectively, these results suggest that some of the antibodies, at least best-in-class antibodies like XGv347, from the repertoire elicited by a 3-dose vaccination regimen retain therapeutic potential against currently circulating VOCs.

Discussion

The ongoing pandemic has witnessed frequent occurrences of SARS-CoV-2 variants that increase transmissibility and reduce potency of vaccine-induced and therapeutic antibodies [4,30]. More recently, there has been unprecedented concern that the Omicron variant has significantly increased antibody escape breadth due to newly occurred and accumulated mutations in the key epitopes of most neutralizing antibodies. Alarmingly, Omicron nearly ablates the neutralization activity of most FDA approved antibody drugs, including LY-CoV555, LY-CoV016, REGN10933, REGN10987,

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

AZD8895 and AZD1061 [2]. These issues raise an urgent need to develop next-generation antibody-based therapeutics that can broadly neutralize these variants, as well as future variants of concern. Our previous study revealed that the regimen of 3-dose vaccination (0, 1, 7 months) of inactivated vaccine leads to an improved immunity response with significantly enhanced neutralizing breadth via ongoing antibody somatic mutation and memory B cell clonal turnover [7,31]. Correlated with this, one subset of highly potent neutralizing antibodies with broad activities (IC_{50} < 0.2 μg/ml) against all circulating VOCs, including Omicron, were present in at least four individuals who had received three doses of inactivated ancestral SARS-CoV-2 vaccine. Some, but not limited to these of this subset antibodies protected against Beta and Omicron infections in mice. Furthermore, our structural and functional analyses revealed that a newly occurred mutation, G446S, might act as a critical antibody escape site, conferring greater resistance to one major class of antibodies bound at the right shoulder of RBD via altering microenverionments at the S-NAb binding interface.

In addition to evading currently available antibody therapeutics, the Omicron variant can diminish the efficacy of all clinically approved vaccines, including the mRNA vaccines and inactivated vaccines [30,32]. There is an ongoing debate about whether the immune responses can be fine-turned to the Omicron variant by boosting with a tweaked (Omicron-based) vaccine. A major hurdle for this approach is the "original antigenic sin", a phenomenon documented in some other infectious diseases, including flu [33]. The presence of a subset of antibodies with broad neutralizing activities against all circulating VOCs in memory B-derived antibody repertoire from the 3-dose vaccinees suggests a possibility that selective and expeditious recall of humoral responses might be elicited via the Omicron/future variants infection, conferring to a secondary protection directed by memory etched in the immune system. Further studies are warranted to examine the advantages and disadvantages of booster shots of an Omicron-specific vaccine or simply administration of a booster with the original vaccines. Lastly the identification and characterization of broadly protective antibodies against all circulating VOCs will aid in the development of universal vaccination strategies against sarbecoviruses.

Online content Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

of author contributions and competing interests; and statements of data and code availability are available at [Article DOI].

Received 22 December 2021; accepted 20 January 2022

1 Carreno, J. M. et al. Activity of convalescent and vaccine serum against a B. 1.1. 529 variant SARS-CoV-2 isolate. nature, doi:https://doi.org/10.1038/d41586-021-03846-z (2021).

2 Cao, Y. R. et al. B. 1.1. 529 escapes the majority of SARS-CoV-2 neutralizing antibodies of diverse epitopes. nature (2021).

3 Mlcochova, P. et al. SARS-CoV-2 B. 1.617. 2 Delta variant replication and immune evasion. Nature, 1-6 (2021).

4 Wang, G.-L. et al. Susceptibility of circulating SARS-CoV-2 variants to neutralization. New England Journal of Medicine (2021).

5 Altmann, D. M., Boyton, R. J. & Beale, R. Immunity to SARS-CoV-2 variants of concern. Science 371, 1103-1104 (2021).

6 Karim, S. S. A. & Karim, Q. A. Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic. The Lancet (2021).

7 Wang, K. et al. A third dose of inactivated vaccine augments the potency, breadth, and duration of anamnestic responses against SARS-CoV-2. medRxiv (2021).

8 Hastie, K. M. et al. Defining variant-resistant epitopes targeted by SARS-CoV-2 antibodies: A global consortium study. Science 374, 472-478 (2021).

9 Yuan, M. et al. Structural and functional ramifications of antigenic drift in recent SARS-CoV-2 variants. Science (2021).

10 Zhang, J. et al. Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant. Science, eabl9463 (2021).

11 Saito, A. et al. Enhanced fusogenicity and pathogenicity of SARS-CoV-2 Delta P681R mutation. Nature, 1-10 (2021).

12 Mbaeyi, S. et al. The Advisory Committee on Immunization Practices' Interim Recommendations for Additional Primary and Booster Doses of COVID-19 Vaccines—United States, 2021. Morbidity and Mortality Weekly Report 70, 1545 (2021).

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

13 Garcia-Beltran, W. F. et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. Cell, doi:https://doi.org/10.1038/d41586-021-03846-z (2021).

14 Gao, Q. et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science 369, 77-81 (2020).

15 Hasan, S. A. W. Interim statement on booster doses for COVID-19 vaccination. Update 4 (2021).

16 Wang, Z. et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. Nature 592, 616-622 (2021).

17 Lv, Z. et al. Structural basis for neutralization of SARS-CoV-2 and SARS-CoV by a potent therapeutic antibody. Science 369, 1505-1509 (2020).

18 Yao, H. et al. Rational development of a human antibody cocktail that deploys multiple functions to confer Pan-SARS-CoVs protection. Cell research 31, 25-36 (2021).

19 Barnes, C. O. et al. SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. Nature 588, 682-687 (2020).

20 Dejnirattisai, W. et al. The antigenic anatomy of SARS-CoV-2 receptor binding domain. Cell 184, 2183-2200. e2122 (2021).

21 Tong, P. et al. Memory B cell repertoire for recognition of evolving SARS-CoV-2 spike. Cell 184, 4969-4980 e4915, doi:10.1016/j.cell.2021.07.025 (2021).

22 Wang, L. et al. Ultrapotent antibodies against diverse and highly transmissible SARS-CoV-2 variants. Science 373, eabh1766 (2021).

23 Park, Y.-J. et al. Antibody-mediated broad sarbecovirus neutralization through ACE2 molecular mimicry. Biorxiv (2021).

24 Cameroni, E. et al. Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. bioRxiv (2021).

25 Martinez, D. R. et al. A broadly cross-reactive antibody neutralizes and protects against sarbecovirus challenge in mice. Science translational medicine, eabj7125 (2021).

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

26 Zhu, L. et al. Double lock of a potent human therapeutic monoclonal antibody against SARS-CoV-2. National Science Review 8, nwaa297 (2021).

27 Hansen, J. et al. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. Science 369, 1010-1014 (2020).

28 Chen, Q. et al. Transient acquisition of cross-species infectivity during the evolution of SARS-CoV-2. National science review 8, nwab167 (2021).

29 Bao, L. et al. The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. Nature 583, 830-833 (2020).

30 Wilhelm, A. et al. Reduced Neutralization of SARS-CoV-2 Omicron Variant by Vaccine Sera and monoclonal antibodies. medRxiv (2021).

31 Wang, Z. et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. Nature 595, 426-431 (2021).

32 Gardner, B. J. & Kilpatrick, A. M. Estimates of reduced vaccine effectiveness against hospitalization, infection, transmission and symptomatic disease of a new SARS-CoV-2 variant, Omicron (B. 1.1. 529), using neutralizing antibody titers. medRxiv (2021).

33 Biswas, A., Chakrabarti, A. K. & Dutta, S. Current challenges: from the path of "original antigenic sin" towards the development of universal flu vaccines: Flu vaccine efficacy encounters significant hurdles from pre-existing immunity of the host suggesting assessment of host immunity before vaccination. International reviews of immunology 39, 21-36 (2020).

34 Cui, Z. et al. Structural and functional characterizations of altered infectivity and immune evasion of SARS-CoV-2 Omicron variant. bioRxiv (2021).

Methods

Facility and ethics statements

All procedures associated with SARS-CoV-2 live virus were approved by the Animal experiment Committee Laboratory Animal Center, Beijing Institute of Microbiology

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

and Epidemiology with an approval number of IACUC-IME-2021-022 and performed in Biosafety Level 3 (BSL-3) laboratories in strict accordance with the recommendations in the Guide for Care and Use of Laboratory Animals. The procedures about human participants were approved by the Ethics Committee (seal) of Beijing Youan Hospital, Capital Medical University with an approval number of LL-2021-042-K. All participants were provided written informed consent.

Viral stock and cell lines

SARS-CoV-2 WT strain CN01 was originally isolated from a patient during the early phase of COVID-19 endemic in China. SARS-CoV-2 variant of concern (VOC) Beta (B.1.351 lineage) strain GDPCC was isolated in a patient from South Africa and an Omicron (B.1.1.529 lineage) strain was isolated from a patient in Hong Kong and now preserved in SinoVac Biotech Ltd. All virus strains were first purified by standard plaque assay as previously described[14] and then inoculated into Vero cells (CCL-81) grown to 95% in 10% fetal bovine serum (FBS) supplemented Dulbecco's minimal essential medium (DMEM) for amplification.

Human sera samples

The serum samples were obtained from healthy volunteers who had no history of COVID-19 and were verified by PCR and serological assay and received two doses or three doses of CoronaVac (Sinovac) inactivated vaccine specific against SARS-COV-2. The whole study was conducted in accordance with the requirements of Good Clinical Practice of China.

Authentic virus neutralization assay

The serum samples were first incubated at 56 ℃ for 30 min for inactivation. The heat-treated samples or monoclonal antibodies (mAbs) were subject to seral dilution from 1: 4 or 50 μg/ml with DMEM in two-fold steps and mixed with a virus suspension containing 100 $TCID_{50}$ at 36.5℃ for 2h, after which, the mixtures were added to wells seeded with confluence Vero cells and incubated at 36.5℃ for another 5 days in a humidified 5% CO_2 cell incubator. After that, the cytopathic effect (CPE) of each well was observed under microscopes by three different individuals and the related dilutions and concentrations were recorded and used for the titration of samples tested by the method of Reed-Muench[14].

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

Pseudovirus neutralization assay

The pseudotyped viruses bearing the S protein were generated, aliquoted and restored as previously described[18]. Briefly, 293T cells were first transfected with the plasmid embedded with the S gene of WT or VOC/VOI (Alpha, Beta, Gamma, Delta, Lamda and Omicron) SARS-CoV-2. The transfected 293T cells were infected with VSV G pseudotyped virus (G*ΔG-VSV) at a multiplicity of infection (MOI) of 4. After incubation for five hours, cells were washed with PBS, and then complete culture medium was added. After another 24 hours, the SARS-CoV-2 pseudoviruses were produced and harvested. For the *In vitro* pseudotyped virus neutralization assay, the plasma samples or antibodies were diluted in DMEM starting from 1:10 or 10 μg/ml with 6 additional threefold serial dilutions, each of which were mixed with the harvested pseudovirus and incubated at 37 °C for 1h. After that, the mixtures were added to Huh-7 cells and placed back for incubation for another 24 hours. Then, the luciferase luminescence (RLU) of each well was measured with a luminescence microplate reader. The neutralization percentage was calculated as following: Inhibition (%) = [1- (sample RLU- Blank RLU) / (Positive Control RLU-Blank RLU)] (%). Antibody neutralization titers were presented as 50% maximal inhibitory concentration (IC_{50}).

Protein expression and purification

The sequences of VOC Omicron full-length S protein (residues 1-1208), receptor-binding domain (RBD) (residues 319-541) and N-terminal domain (NTD) (residues 1-304) were modified from the plasmids encoding the S, RBD and NTD of WT SARS-COV-2 (GenBank: MN908947) in our lab by overlapping PCR. In additional to the reported mutations (A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F) on Omicron, the proline substitutions at 817, 892, 899, 942, 986 and 987, 'GSAS' substitutions at the S1/S2 furin cleavage site (residues 682-685) and a C-terminal T4 foldon trimerization domain were also introduced in the Omicron S construct to stabilize the trimeric conformation of S protein. For protein expression, the plasmids of these proteins were transiently transfected into HEK293F cells grown in suspension at 37 °C in an incubator supplied with 8% CO_2, rotating at 130 rpm. The cell supernatants were

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

harvested and concentrated three days post-transfection, and further purified by affinity chromatography using resin attached with streptavidin and size-exclusion chromatography (SEC) using a Superose 6 10/300 column (GE Healthcare Life Sciences) equilibrated with the buffer containing 20 mM Tris-HCl, pH 8.0, and 200 mM NaCl.

Single memory B cell isolation and sequencing

PBMCs were separated from the whole-blood samples obtained from four volunteers using Histopaque (Sigma) gradient centrifugation. After washing with Hank's balanced salt solution (HBSS) (Solarbio) for three times, the cells were aliquoted and stored in liquid nitrogen in the presence of FBS and DMSO. For single memory B cell sorting, stored PBMCs were thawed and incubated with CD19 MicroBeads (Miltenyi Biotec) to screen out CD19+ B lymphocytes, which were then incubated with human Fc block (BD Biosciences), anti-CD20-PECy7 (BD1113 Biosciences), S-ECD-PE, and S-ECD-APC. The single memory B cells (CD20-1114 PECy7+ S-ECD-PE+ S-ECD-APC+) were further sorted into 96-well plates using a FACSAria II (BD Biosciences), and followed by sequencing and cloning as previously described[35].

Antibody expression and Fab generation

The selected 323 antibodies were subjected to gene codon optimization and construction with a plasmid encoding human IgG1 Fc as described previously [7]. Then the clones were transiently transfected into mammalian HEK293F cells and incubated for 5 days in a 5% CO_2 rotating incubator at 37°C for antibody expression, which were further purified using protein A and dialyzed into Phosphate Buffered Saline (PBS). The purified mAbs XGv265, XGv282, XGv289 and XGv347 were then processed to obtain their Fab fragments using the Pierce FAB preparation kit (Thermo Scientific) as described previously [36]. Briefly, the samples were first applied to desalination columns to remove the salt and the flow-throughs were collected and incubated with papain that was attached with beads to cleave Fab fragments from the whole antibodies for 5 hours at 37°C. After that, the mixtures were transferred into Protein A columns and the flow-throughs, i.e., the Fab fragments were collected and dialyzed into PBS (ThermoFisher, catalog #10010023).

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

Bio-layer interferometry

Bio-layer interferometry (BLI) experiments were run on an Octet Red 384 machine (Fortebio). To measure the binding affinities of mAbs, monoclonal antibodies were immobilized onto Protein A biosensors (Fortebio) and the threefold serial dilutions of WT RBD, Alpha RBD (ACROBiosystems, Cat No. SPD-C52Hn), Beta RBD (ACROBiosystems, Cat No. SPD-C52Hp), Gamma RBD (ACROBiosystems, Cat No. SPD-C52Hr), Delta RBD (ACROBiosystems, Cat No. SPD-C52Hh) and Omicron RBD (ACROBiosystems, Cat No. SPD-C522e) in PBS were used as analytes. Data were then analyzed using software Octet BLI Analysis 12.2 (Fortebio) with a 1:1 fitting model. For the competitive assay by BLI, SARS-CoV-2 WT RBD tagged with His (ACROBiosystems, Cat No. SPD-C52H3) was loaded on NTA biosensors, which were pre-equilibrated in the buffer for at least 1 min. The loaded biosensors were immersed with the first mAb for 300 s, followed by addition of the second mAb for another 300 s. Data obtained were also analyzed by Octet BLI Analsis 12.2.

ELISA assays

To evaluate whether the given mAbs could block the interaction between human ACE2 (hACE2) and RBD, ACE2 competition ELISA was performed by using the SARS-CoV-2 (B.1.1.529) Inhibitor Screening Kit (ACROBiosystems, Cat No. EP-115) according to the recommended protocol. Briefly, each of the 10 two-fold dilution series of mAbs (starting dilution of 25 μg/ml) and 0.8 μg/ml of HRP-conjugated SARS-CoV-2 RBD were added into the ELISA plate wells which are pre-coated with hACE2 protein. After incubation at 37 °C for 1 hour, the plates were washed three times with PBST (0.1% Tween) and the colorimetric signals were developed by addition of 3, 3', 5, 5'-tetramethylbenzidine TMB (Thermo Fisher) for 10 min. The reaction was stopped by addition of 50 μL of 1M H2SO4. The absorbance was measured at 450 nm with an ELISA microplate reader. For each mAb, a blank control with no mAb was added for inhibition calculation. The area under the curve (AUC) of each mAb were determined using Prism V8.0 (GraphPad). For competitive ELISAs to identify the domain of a given mAb, 96-well plates were first coated with RBD (2 μg/ml) and then blocked with 2% BSA in PBS. After incubation with the reference mAbs, the blocking antibody (15 μg/ml), the wells were followed by directly adding the second biotinylated antibodies (0.25 μg/ml). Streptavidin-HRP (BD Biosciences)

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

was then added for detection. Samples with no first antibody were used as a negative control for normalization.

Cryo-EM sample preparation, data collection

The purified S protein was mixed with each of the Fab fragments of XGv265, XGv282, XGv289 or XGv347 with a molar ratio of 1: 1.2 for 10 s ice incubation, and then dropped onto the pre-glow-discharged holey carbon-coated gold grid (C-flat, 300-mesh, 1.2/1.3, Protochips In.), blotted for 7 seconds with no force in 100% relative humidity and immediately plunged into the liquid ethane using Vitrobot (FEI). Cryo-EM data sets of these complexes were collected at 300 kV with an FEI Titan Krios microscope (FEI). Movies (32 frames, each 0.2 s, total dose of 60 e^{-} $Å^{-2}$) were recorded using a K3 Summit direct detector with a defocus range between 1.5-2.7 μm. Automated single particle data acquisition was carried out by SerialEM, with a calibrated magnification of 22,500 yielding a final pixel size of 1.07 Å.

Cryo-EM data processing

A total of 3,752, 2,631, 3,955 and 5,014 micrographs of S-XGv265-complex, S-XGv282-complex, S-XGv289-complex and S-XGv347-complex, respectively were recorded and subjected to beam-induced motion correction using motionCorr in Relion 3.0 package [37]. The defocus value of each image was calculated by Gctf. Then, 1,302,103, 756,508, 2,332,045 and 2,320,416 particles of the S-XGv265-complex, S-XGv282-complex, S-XGv289-complex and S-XGv347-complex, respectively were picked and extracted for reference-free 2D alignment by cryoSPARC [38], based of which, 422,083, 190,154, 837,832 and 614,852 particles were selected and applied for 3D classification by Relion3.0 for S-XGv265-complex, S-XGv282-complex, S-XGv289-complex and S-XGv347-complex, respectively with no symmetry imposed to produce the potential conformations for the complexes. Afterwards, the candidate model for each complex was selected and processed by non-uniform auto-refinement and postprocessing in cryoSPARC to generate the final cryo-EM density for S-XGv265-complex, S-XGv282-complex, S-XGv289-complex and S-XGv347-complex. To improve the resolution of the interface between RBD and mAbs, the block-based reconstruction was performed to obtain the final resolution of the focused interfaces which contained the interfaces of RBD and mAbs investigated here as described previously [39]. The resolution of each structure was determined on the basis of the gold-standard Fourier shell correlation (threshold = 0.143) and evaluated by

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

ResMap. All dataset processing is shown in Extended Data Fig. 3 and also summarized in Extended Data Table 2.

Model fitting and refinement

The atomic models of the complexes were generated by first fitting the chains of the native apo SARS-CoV-2 S trimer (PDB number of 6VYB) and Fabs (PDB number of 7LSS and 7CZW for XGv265, 5MES and 5VAG for XGv282, 6UDA and 7MEG for XGv289 as well as 7E3K for XGv347) into the cyo-EM densities of the final S-Fab-complexes described above by Chimera, followed by manually adjustment and correction according to the protein sequences and densities in Coot, as well as real space refinement using Phenix. Details of the refinement statistics of the complexes are summarized in Extended Data Table 2.

MD simulation and ΔG estimation

Model of SARS-CoV-2 WT RBD in complex with XGv265, XGv282, XGv289 and XGv347 were generated in Chimera by superimposition of WT RBD and cryoEM structure of Omicron RBD in complex with the four antibodies. Before molecular dynamics, all models were checked by WHAT IF Web Interface (https://swift.cmbi.umcn.nl/servers/html/index.html) to model missing sidechains and remove atomic clashes. After that, the structure was simulated by GROMACS-2021. Briefly, we used OPLS force field with TIP3P water model to prepare the dynamic system and add Na+ and Cl- ions to make the system electrically neutralized. Then, the system was subjected to energy minimization using the steepest descent algorithm until the maximum force of 1,000 kJ mol-1 has been achieved. NVT ensemble via the Nose-Hoover method at 300 K and NPT ensemble at 1 bar with the Parinello-Rahman algorithm were employed successively to make the temperature and the pressure equilibrated, respectively. Finally, a MD production runs of 100 ns were performed starting from random initial velocities and applying periodic boundary conditions. The non-bonded interactions were treated using Verlet cut-off scheme, while the long-range electrostatic interactions were treated using particle mesh Ewald (PME) method. The short-range electrostatic and van der Waals interactions were calculated with a cut-off of 12 Å. Average structure of the four complexes were generated using the last 10 ns frames and ΔG between the antibodies and RBD was estimated in ROSETTA by InterfaceAnalyzer. Atomic_burial_cutoff,

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

sasa_calculator_probe_radius and interfaces_cutoff values were set to 0.01, 1.4 and 8.0 respectively.

In vivo protection against SARS-CoV-2 Beta and Omicron variants challenge in mice

The *in vivo* protection efficacies of single antibody or antibody cocktails were assessed by using a newly established mouse model based on a SARS-CoV-2 Beta variant strain [28]. Briefly, groups of 8-month-old female BALB/c mice were infected with 1×10^4 PFU of SARS-CoV-2 Beta variant strain, then infected mice were treated intraperitoneally with a single dose of different antibodies or antibody cocktails (5 mg/kg) at 1 hour after infection. The protection efficacy of XGv347 was also assessed by using 10-week-old K18-hACE2 mice, each challenged with 1×10^2 $TCID_{50}$ of Omicron strain. And two 2 hours post infection, mice were intraperitoneally treated with a single dose of XGv347 at 30 mg/kg or the same volume of PBS as control. The lung tissues of mice from both two groups were collected at 5 dpi for viral RNA loads assay and pathological examination. All mice were randomly allocated in each group.

Viral burden determination

Viral burden in lung from mice were measured as described previously [17]. Briefly, lung tissue homogenates were clarified by centrifugation and viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen). Viral sgRNA quantification in each tissue sample was performed by quantitative reverse transcription PCR (RT-qPCR) targeting the S gene of SARS-CoV-2. RT-qPCR was performed using One-Step PrimeScript RT-PCR Kit (Takara).

Histology, and RNA in situ hybridization (RNA ISH)

Lung tissues from mice were fixed with perfusion fixative (formaldehyde) for 48 h, and embedded in paraffin according to standard histological assays. For histopathology, lung tissues were stained with hematoxylin and eosin (H&E). Images were captured using Olympus BX51 microscope equipped with a DP72 camera. For RNA ISH assays were performed with an RNAscope 2.5 (Advanced Cell Diagnostics) according to the manufacturer's instruction. Briefly, formalin-fixed paraffin-embedded tissue sections of 5 μm were deparaffinized by incubation for 60 min at 60 °C. Endogenous peroxidases were quenched with hydrogen peroxide for 10 min at room temperature. Slides were then boiled for 15 min in RNAscope Target Retrieval Reagents and incubated for 30 min in RNAscope Protease Plus before probe

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

hybridization. The probe targeting 2019-nCoV RNA was designed and synthesized by Advanced Cell Diagnostics (catalog no. 848561). Tissues were counterstained with Gill's hematoxylin and visualized with standard bright-field microscopy. Original magnification was 10×.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The atomic coordinates of XGv347 in complex with S trimer (state 1), XGv347 in complex with S trimer (state 2), XGv347 in complex with S trimer (state 3), XGv347-S have been submitted to the Protein Data Bank with accession numbers: 7WEA, 7WEC and 7WEB, respectively. Furthermore, the atomic coordinates of XGv265, XGv282 and XGv289 have been deposited in the protein data bank under accession code 7WE8, 7WE7 and 7WE9, respectively. Cryo-EM density maps in this study have been deposited at the Electron Microscopy Data Bank with accession codes EMD-32444 (state 1), EMD-32446 (state 2) and EMD-32445 (state 3), EMD-32441 (XGv282), EMD-32442 (XGv265), and EMD-32443 (XGv289). To reveal structural details of Fab binding mechanism, the local optimized method was used to optimized data progress and the related atomic models and EM density maps of optimized reconstructions of Fab interaction interface has been deposited under accession code 7WEE (XGv265), 7WED (XGv347), 7WLC (XGv282), 7WEF (XGv289), EMD-32447 (XGv347), EMD-32448 (XGv265), EMD-32581(XGv282), EMD-32449 (XGv289), respectively.

35 Zhou, Y. *et al.* Enhancement versus neutralization by SARS-CoV-2 antibodies from a convalescent donor associates with distinct epitopes on the RBD. *Cell Rep* **34**, 108699, doi:10.1016/j.celrep.2021.108699 (2021).

36 Wang, N. *et al.* Structure-based development of human antibody cocktails against SARS-CoV-2. *Cell research* **31**, 101-103 (2021).

37 Zivanov, J. *et al.* New tools for automated high-resolution cryo-EM structure determination in RELION-3. *elife* **7**, e42166 (2018).

Publisher: NPG; Journal: Nature: Nature; Article Type: Biology article
MS nr: 10.1038/s41586-022-04466-x

38 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nature methods* **14**, 290-296 (2017).

39 Sun, Y. *et al.* Structure-based development of three-and four-antibody cocktails against SARS-CoV-2 via multiple mechanisms. *Cell research* **31**, 597-600 (2021).

Acknowledgements We thank Dr. Xujing Li, Dr. Xiaojun Huang and Lihong Chen for cryo-EM data collection at the Center for Biological imaging (CBI) in Institute of Biophysics for EM work. We also thank Dr. Yuanyuan Chen, Zhenwei Yang and Bingxue Zhou for technical support on SPR. Work was supported by the Strategic Priority Research Program (XDB29010000, XDB37030000), CAS (YSBR-010), National Key Research and Development Program (2020YFA0707500, 2018YFA0900801), Beijing Municipal Science and Technology Project (Z201100005420017) and Ministry of Science and Technology of China (EKPG21-09 and CPL-1233). Xiangxi Wang was supported by Ten Thousand Talent Program and the NSFS Innovative Research Group (No. 81921005). Kang Wang was supported by the Special Research Assistant Project of the Chinese Academy of Medical Sciences.

Author contributions X.W., K.W., C.F.Q., C.Q. and Y.W. conceived, designed and analyzed the whole experiment; Y.H., M.L., Y.L. and Lin W. performed authentic virus neutralizing assay; Z.J., Q.L., X.P., J.W., S.L. and W.H. performed the pseudovirus neutralizing assays; K.W., Y.J., L.Q., P.G., Z.C., Y.C. and K.F. performed plasmid construction, protein and antibody expression. Q.Z. and P.Y. performed the BLI assay. L.B., H.C. and Y.D. performed animal experiments and analyzed the results. K.W., L.W., B.Z., L.C., P.L., W.F. and N.W. performed cryo-EM sample preparation, data collection, and processing. all authors analyzed data; X.W., K.W., C.F.Q., C.Q. and Y.W. wrote the manuscript with input from all co-authors.

Competing interests Y.H., Lin W. and M.L. are employees of Sinovac Biotech Ltd. Y.J., P.G. and Y.C. are employees of Acrobiosystems Inc. Other authors declare no competing interests.

Additional information

Supplementary information is available for this paper at

Correspondence and requests for materials should be addressed to Cheng-Feng Qin, Youchun Wang, Chuan Qin or Xiangxi Wang.

Peer review information *Nature* thanks the anonymous reviewers for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.

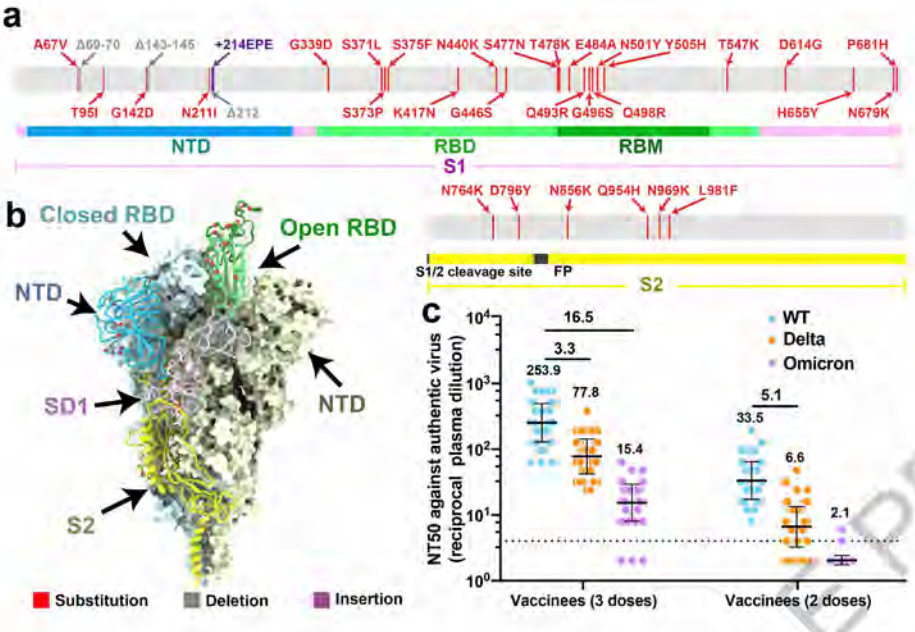


Fig. 1 Evolution and neutralization characteristics of Omicron variant. **a**, A linear representation of Omicron S with mutations marked on. The replacements are marked in red; deletions are in grey and insertions are in purple. **b**, Distribution of mutations of Omicron on the cryo-EM structure of pre-fusion S trimer determined at pH 7.5 (PDB code 7WG6) [34]. The mutations listed in **a** are indicated in the 'up' protomer shown as cartoon with mutated residues highlighted as spheres and colored as in **a**. The RBD, NTD, SD1 and S2 of this subunit are marked with arrow and colored in green, blue, magenta and yellow, respectively; the other two protomers in 'down' state are shown as surface in pale cyan and pale yellow, respectively. **c**, Graph shows the neutralizing antibody response against WT and Omicron SARS-CoV-2 authentic virus for sera from healthy vaccinees who received two doses (n=60 volunteers) or three doses (n=60 volunteers) of Coronavac. Bars and indicated values represent geometric mean of NT_{50} ± SD of technical triplicates. The dotted line represents the detection limit. NT_{50} values less than 4 were plotted as 2. shown above each plot Neutralizing antibody titer fold decline for Delta or Omicron over WT for each group of sera is shown in each of the plots.

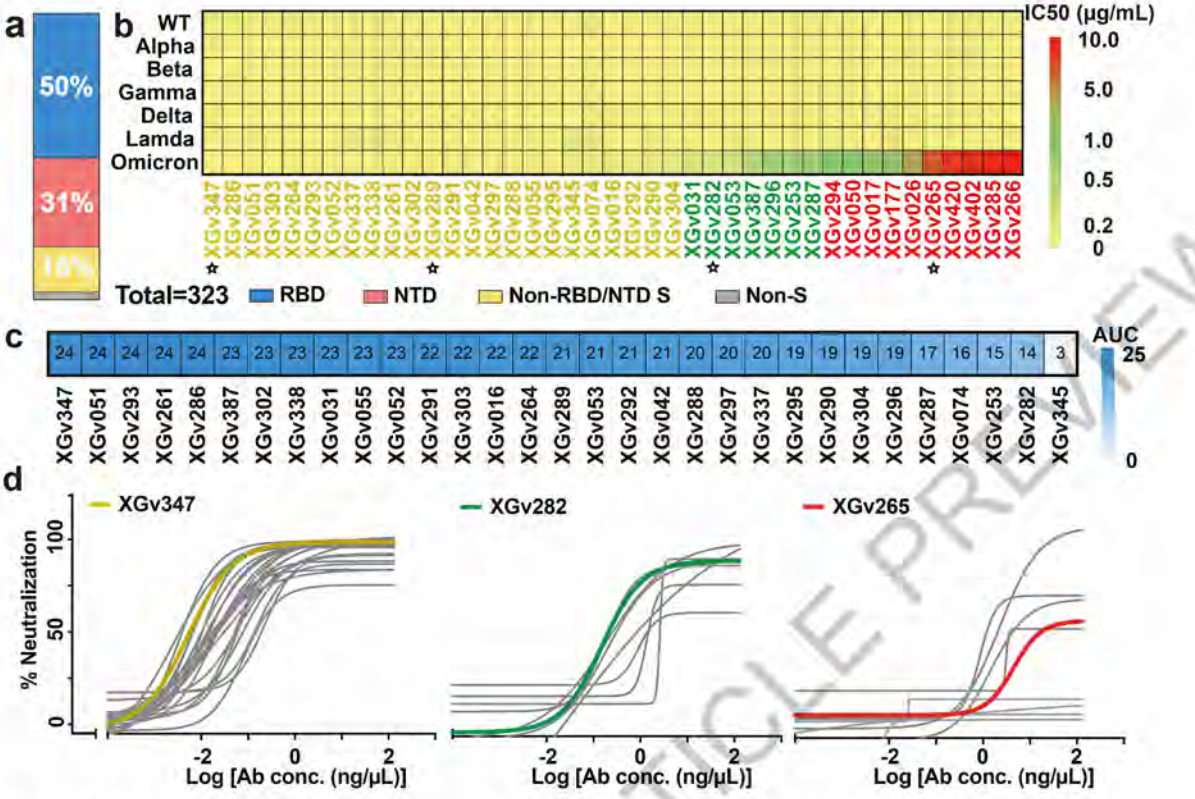


Fig. 2 Characteristics of a subset of broadly neutralizing antibodies from recipients of a booster immunization. a, Vertical slices chart shows the gross binding epitope distribution of mAbs isolated from the individuals who received three doses of inactivated vaccines. Total number of antibodies and the percentage of antibodies that recognize RBD (blue), NTD (red) and S2 domain (yellow) are indicated. **b**, Heatmap representation of 41 selected representative mAbs against pseudotyped viruses with WT or variant SARS-CoV-2 S. The color bar on the right represents the ranges of IC_{50} values for the indicated mAbs against pseudotyped viruses in **c** (yellow: 0.002-0.020 μg/ml; green: 0.020-1.000 μg/ml; red: 1.000-10.000 μg/ml). Antibodies marked with star were selected for structural analysis. **c**, Heatmap with values shown in the form of AUC represents the competition ability between the selected mAbs and hACE2. Color gradient ranging from white (1) to blue (24) is shown on the right represents the competition ability from the weakest to the strongest. **d**, Neutralization curves for the selected 41 antibodies on pseudotyped viruses with the S protein of Omicron variant of concern. Data shown here are three groups of antibodies in correspondence with **b**. yellow - ultrapotent antibodies against all five VOCs, green - highly potent antibodies against other four VOCs, but with median neutralizing activities against Omicron, red - highly potent antibodies against other four VOCs, but with weak neutralizing activities against Omicron. XGv347, XGv282 and XGv265, selected as a representative of each group are highlighted by bold curve in yellow, green, and red, respectively. All experiments were performed in duplicate.

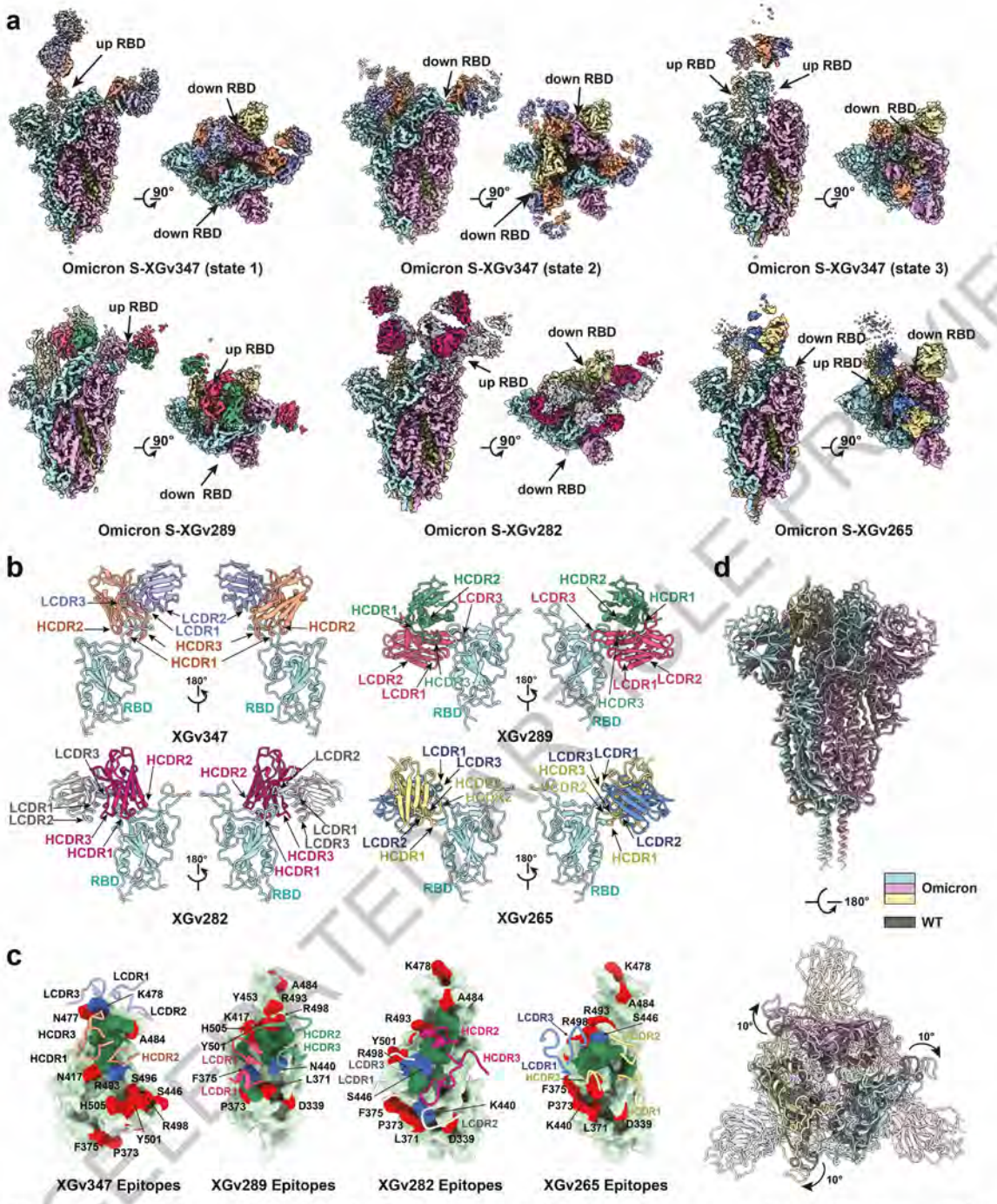


Fig. 3 Structural basis of the broad and potent neutralization of representative antibodies. a. Side view and top view of Cryo-EM maps of SARS-CoV-2 Omicron S trimer in complex with XGv347 (state 1-3), XGv289, XGv282 and XGv265. For XGv347-S-complex, state 1, one up RBD and one down RBD; state 2, three down RBDs; state 3, two up RBDs. **b**. Cartoon representations of the structures of SARS-CoV-2 Omicron-RBD in complex with XGv347 (top-left), XGv289 (top-right), XGv282 (bottom-left) and XGv265 (bottom-right). Two different views for each set are shown to illustrate the binding modes of these four antibodies. RBD is colored in cyan. **c**. Interactions between the four antibodies and SARS-CoV-2 Omicron RBD. The CDRs of the four antibodies that interact with SARS-CoV-2 Omicron RBD are displayed as cartoon over the light green surface of RBD. The mutation sites on RBD of Omicron are colored in red; the epitopes of antibodies are colored in deep green and the overlap of them are colored in blue. Residues of each epitope are marked out in the corresponding regions. d. Superimposition of Omicron onto WT S Trimer. Omicron S trimer is colored in cyan and WT S trimer is colored in yellow.

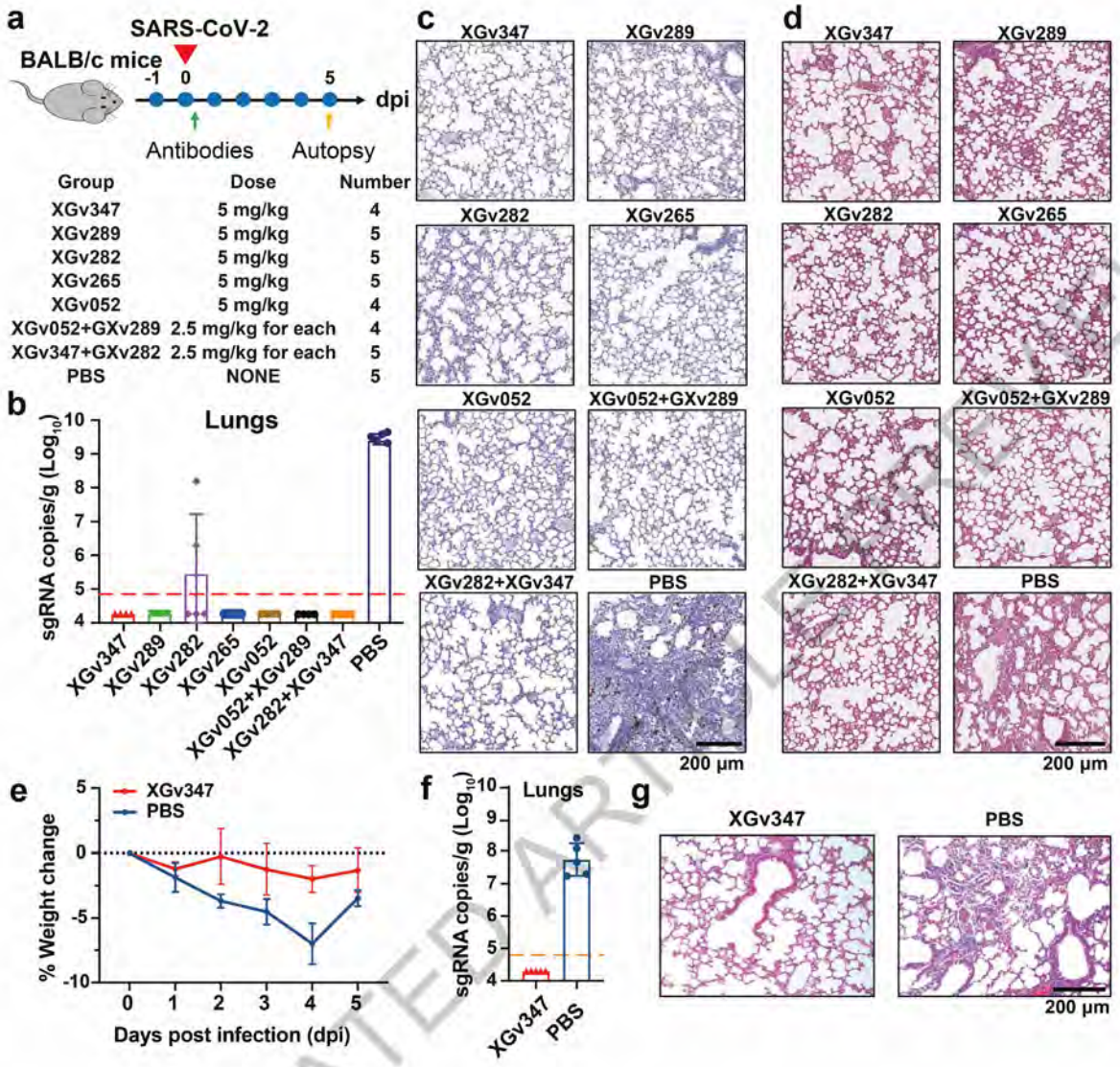
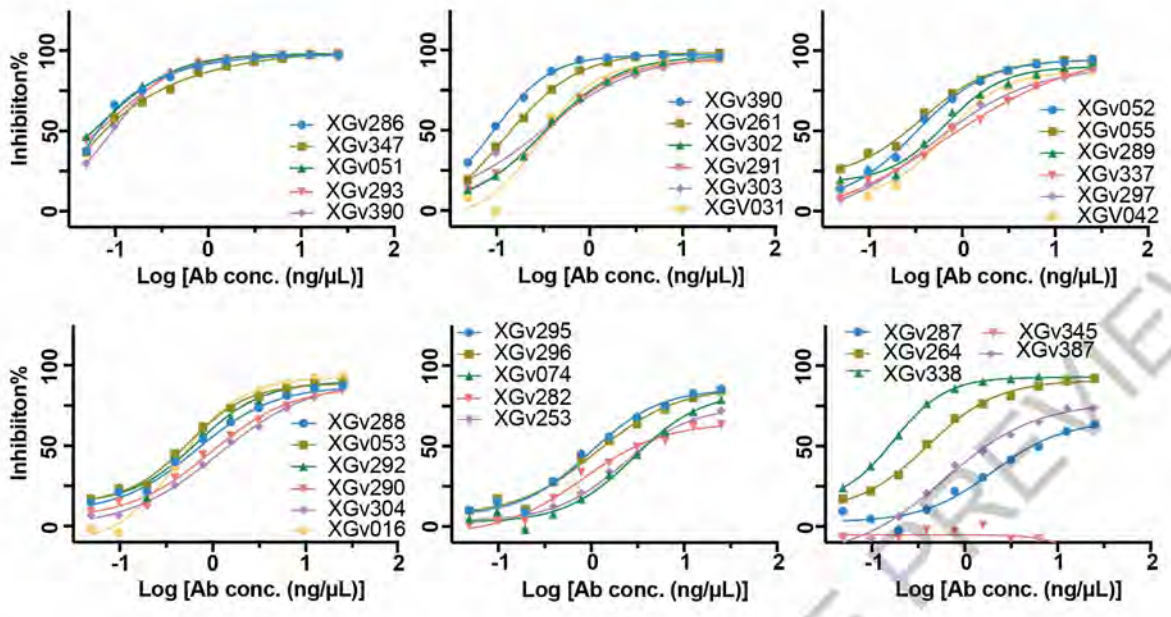
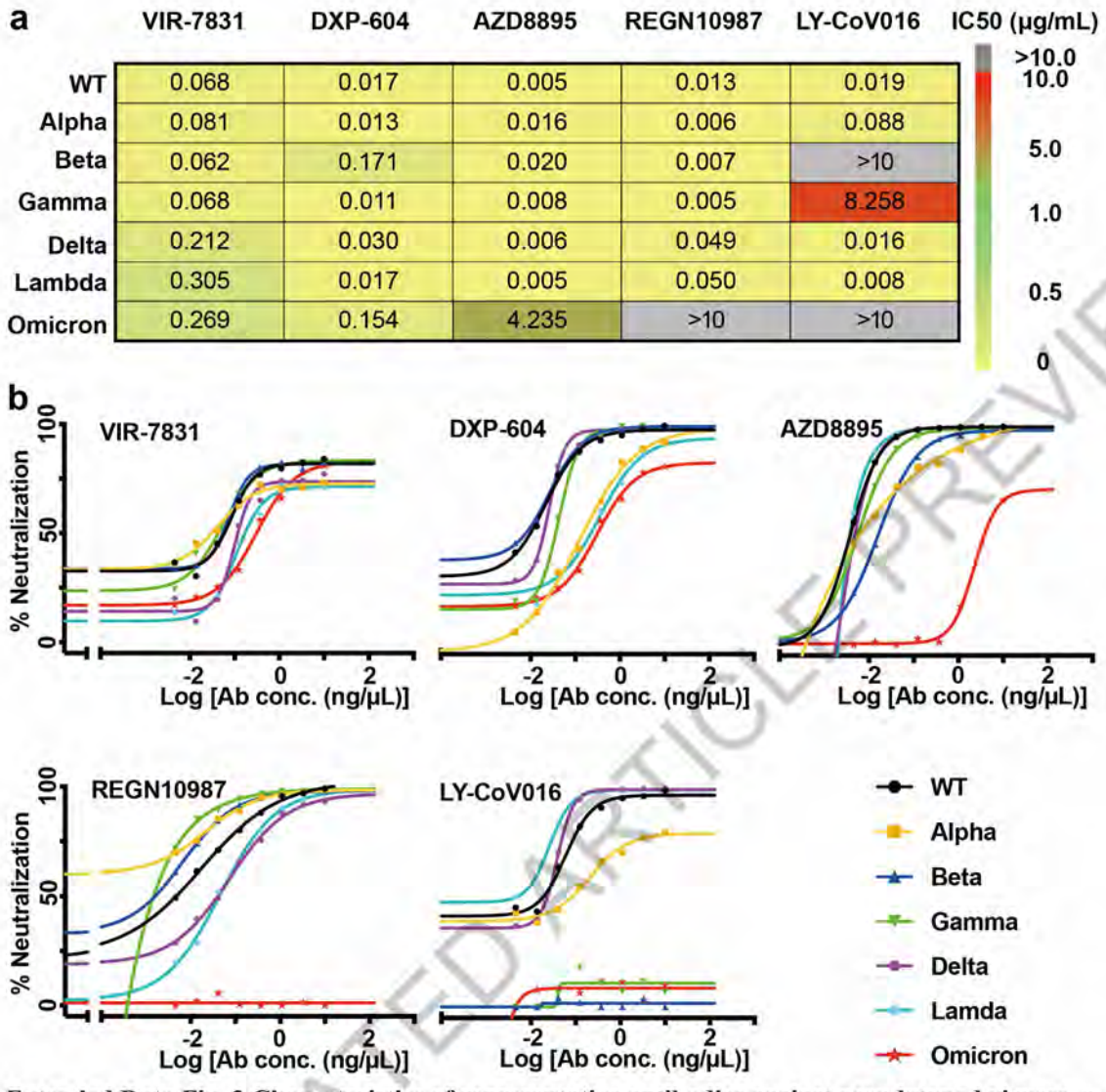


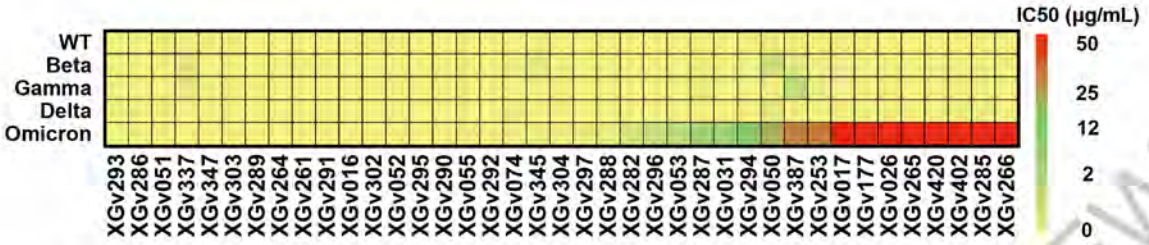
Fig. 4 Protection against SARS-CoV-2 Beta and Omicron variants challenge in mice. a, Experimental design for protection assay against Beta variant challenge. n = 4 mice in XGv347, XGv052, and XGv052 + XGv289 groups; n = 5 mice in other groups. **b to d**, Examination of lung tissues of Beta variant challenged mice collected at 5 dpi for **b**, virus titer, **c**, Immunostaining and **d**, H&E. **b**, Virus RNA loads in the lungs at 5 dpi were measured by RT-qPCR and are expressed as RNA copies per gram. Data are represented as mean ± SD. Dashed line represents limit of detection. **c**, SARS-CoV-2 genome RNA ISH was performed with a SARS-CoV-2 specific probe. Brown-colored staining indicates positive results. Scale bar, 200 μm. **d**, Histopathological analysis of lung samples at 5 dpi. Scale bar: 200 μm. **e to f**, weight change and lung tissues examinations of K18-hACE2 mice challenged with Omicron variant of concern. n = 5 mice in each group. **e**, Weight of each mouse in both groups was monitored and recorded daily post infection. Mean with standard deviation. **f**, Virus RNA loads in the lungs at 5 dpi were also measured as in **b**. Data are represented as mean ± SD**.** Dashed line represents limit of detection. **g**, Histopathological analysis of lung tissues from both two groups. Scale bar, 200 μm. Each micrograph in **c, d and g** is representative of two separate experiments.



Extended Data Fig. 1 Antibody-hACE2 competition ELISA assay. Data shown are the curves of 31 antibodies used to compete with ACE2. All experiments were performed in duplicate.



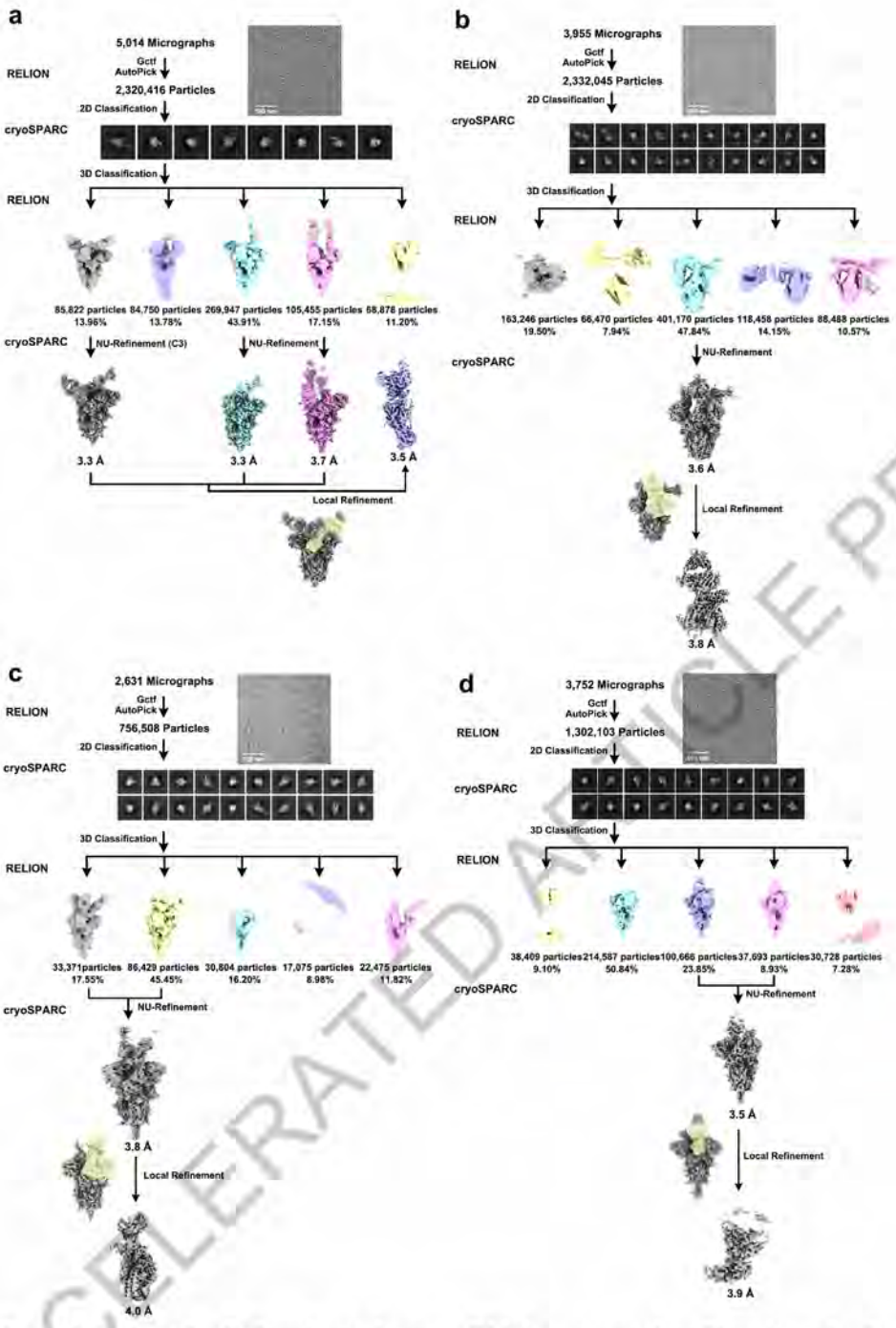
Extended Data Fig. 2 Characteristics of representative antibodies against pseudotyped viruses. a, Heatmap representation of five therapeutic mAbs approved or in clinical trials against pseudotyped viruses with the S proteins of wild-type or variants of concern or interst (Alpha, Beta, Gamma, Delta, Lambda and Omicron). **b,** Neutralization curves for these mAbs in correspondence with **a**. Mean of two experiments is shown.



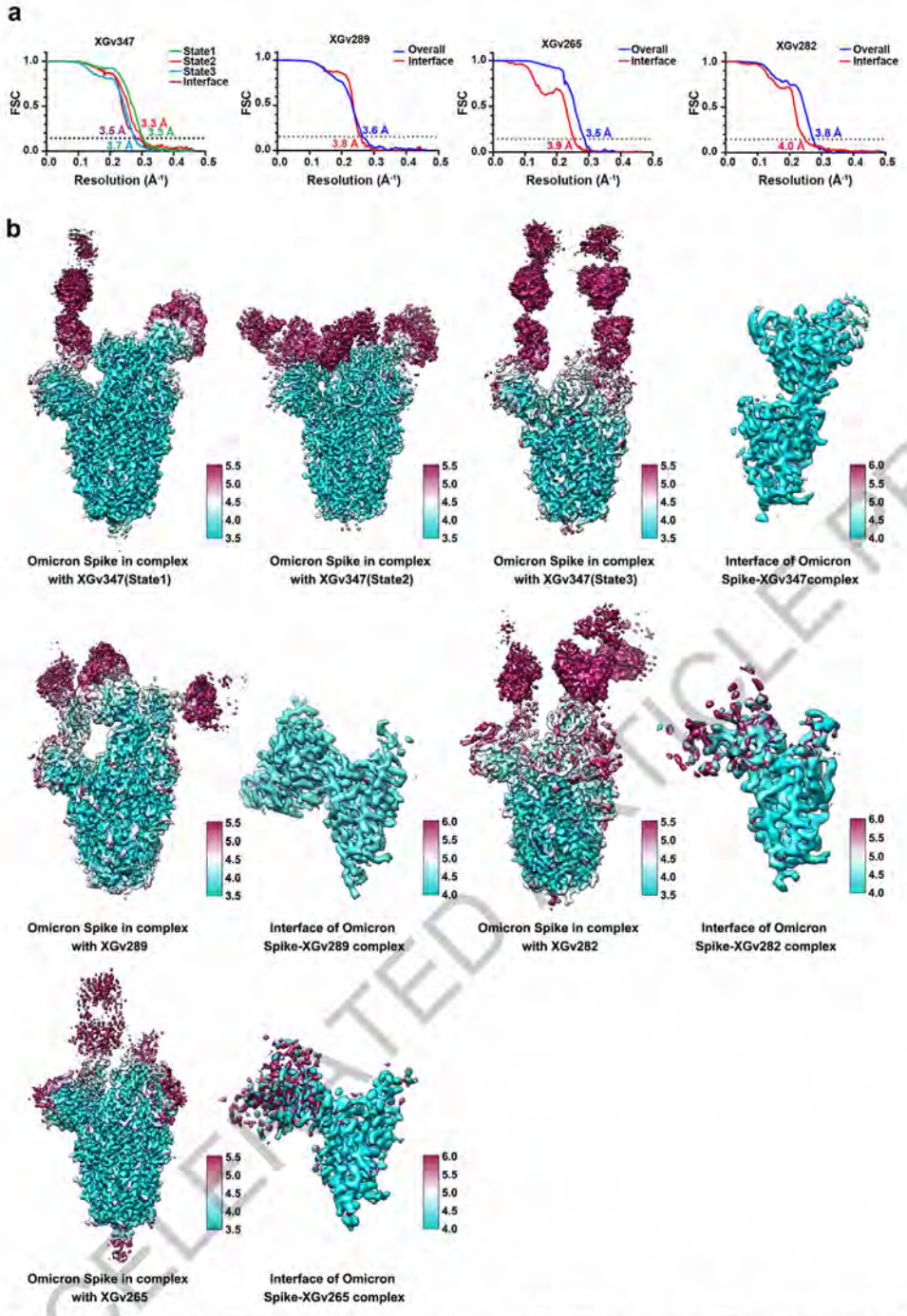
Extended Data Fig. 3 Heatmap representation of representative mAbs against WT and variants of concern. Color bar on the right showed the gradient of IC50 of different antibodies against the authentic WT and variants of concern. All experiments were performed in duplicate.

		Class I				Class II		Class III		Class IV			Class V			Class VI			NC
		XGv013	XGv026	P4A1	XG017	414	H4	XGv031	81**	XGv016	S309	XG014	XGv030	XG025	FC08	XGv004	XG011	A34-2	Fc05
XGv051	1	4	3	3	4	3	9	5	6	94	89	73	71	85	49	325	117	22	101
XGv052	1	3	3	3	3	3	6	4	5	76	55	79	71	86	59	87	66	22	90
XGv053	1	3	5	3	3	3	5	4	7	75	60	76	77	74	57	84	61	22	89
XGv055	1	9	3	3	4	3	9	5	6	89	105	78	83	88	67	321	133	14	95
XGv074	1	5	4	5	5	4	8	7	19	91	61	107	77	89	87	112	80	48	97
XGv253	1	3	3	4	3	3	7	5	5	96	65	80	86	86	81	163	130	145	81
XGv261	1	5	4	3	4	3	9	8	12	91	79	78	71	75	78	272	118	25	94
XGv302	1	3	3	3	4	3	8	7	11	74	73	90	81	90	86	238	144	44	82
XGv303	1	4	4	3	4	3	7	8	13	85	78	92	86	96	89	254	148	47	69
XGv304	1	4	4	4	4	3	7	10	19	89	81	98	83	101	89	237	128	46	99
XGv387	1	6	6	5	4	5	8	15	28	80	63	75	67	60	95	95	71	23	145
XGv177	2	13	18	5	5	5	9	12	27	52	54	67	57	60	87	79	69	24	93
XGv285	2	69	38	78	84	7	10	85	94	43	71	14	72	84	96	94	81	161	107
XGv286	2	41	78	69	73	3	5	80	91	4	88	5	83	77	96	70	90	143	88
XGv287	2	115	81	78	81	15	41	118	104	69	106	32	79	96	96	164	100	133	89
XGv288	2	66	73	69	76	3	7	94	81	5	72	5	69	81	87	77	76	114	91
XGv290	2	65	73	51	76	4	11	81	82	9	60	6	54	77	94	71	69	97	101
XGv291	2	46	72	69	76	3	6	80	83	5	62	4	83	72	92	68	71	127	85
XGv292	2	69	77	62	79	3	5	74	86	7	97	5	81	77	86	75	63	107	86
XGv293	2	42	84	68	80	3	6	88	85	4	79	4	82	82	92	79	73	104	90
XGv294	2	64	78	71	81	3	6	87	88	8	61	5	76	73	87	79	72	96	88
XGv295	2	94	86	74	79	5	11	125	91	9	98	5	91	96	101	280	102	104	106
XGv297	2	57	81	75	76	3	7	101	85	4	76	4	79	72	85	73	79	131	91
XGv338	2	88	85	82	83	3	5	5	4	82	65	47	80	61	5	95	78	100	99
XGv347	2	3	3	3	3	3	7	4	5	88	84	86	96	91	85	256	149	131	97
XGv402	2	9	12	5	7	4	8	23	36	89	63	85	69	73	89	92	74	39	107
XGv420	2	131	88	88	93	3	9	120	51	25	12	5	75	46	64	318	126	57	100
XGv337	3	109	66	77	82	3	9	8	5	79	64	38	67	53	5	277	129	132	94
XGv264	4	63	79	73	84	3	6	93	86	4	5	4	86	72	79	82	84	134	86
XGv265	4	73	77	62	83	3	6	87	86	16	7	5	74	64	80	74	76	108	89
XGv266	4	73	78	87	87	3	5	91	80	12	6	4	85	64	83	86	76	127	86
XGv282	4	81	79	105	77	3	5	83	10	5	43	4	93	76	11	70	89	106	88
XGv289	4	57	78	74	72	3	7	91	77	8	71	5	87	72	82	67	86	143	87
XGv296	4	51	74	64	67	3	6	86	87	5	79	5	80	64	85	67	72	122	87
XGv345	4	88	95	87	92	3	7	97	31	22	7	4	76	12	14	248	132	121	65

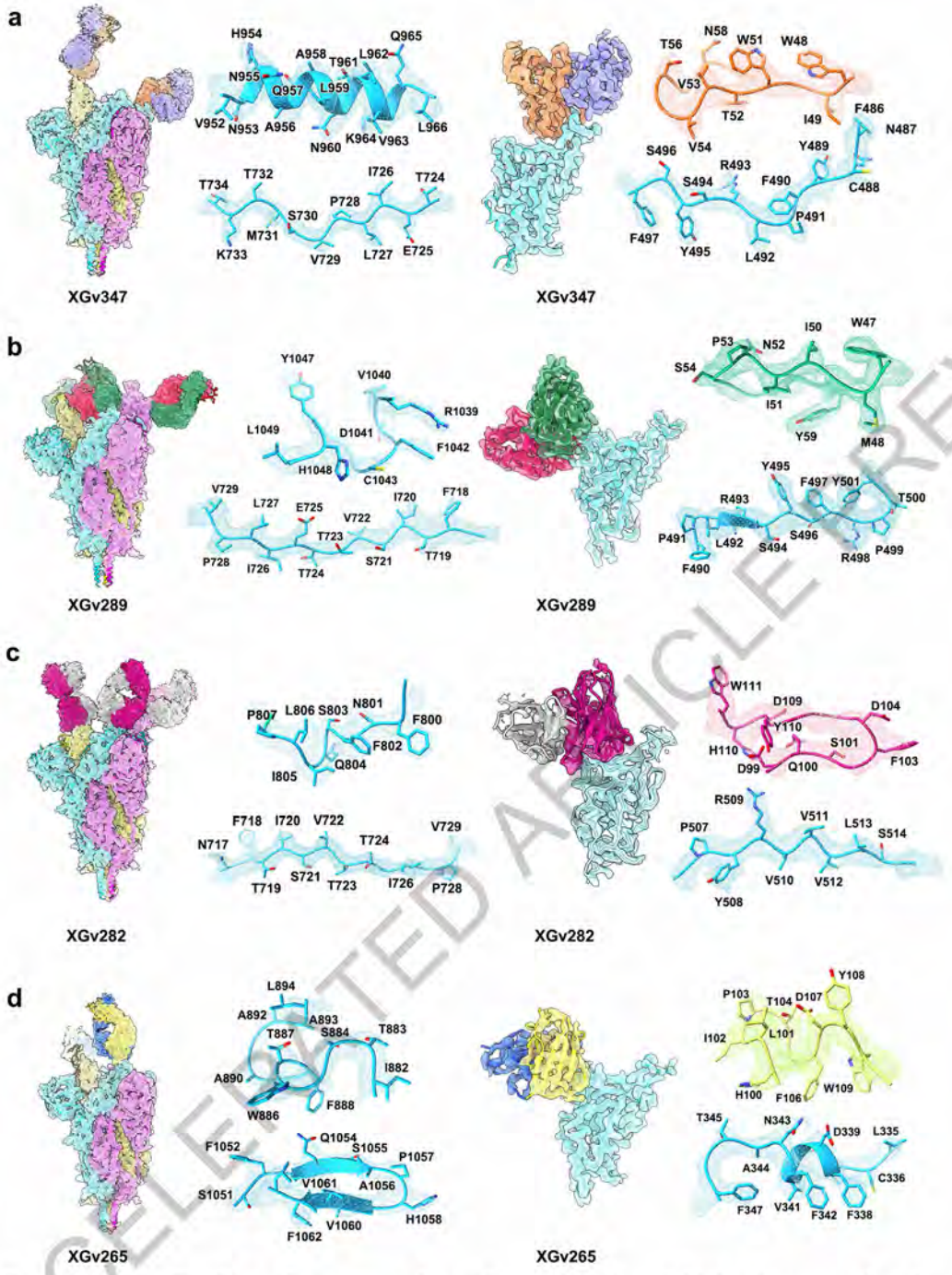
Extended Data Fig. 4 Data sheets of ELISA assay of representative mAbs against Omicron RBD. Different Classes of mAbs (Class I-VI) are colored by yellow, green, red, blue, brown and magenta, respectively. Values are filled with black (>75), grey (50-75), silver (25-50) and white (<25). Each data is the mean of three values from three independent experiments.



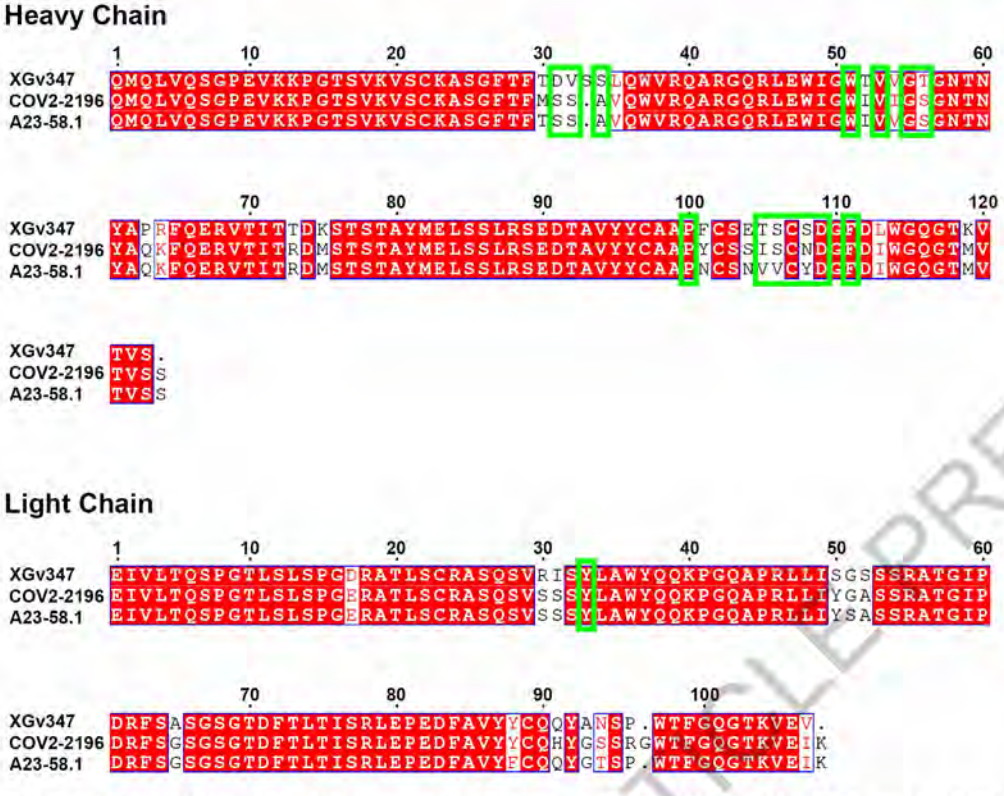
Extended Data Fig. 5 Flowcharts for cryo-EM data processing. Flowcharts for Omicron S protein in complex with **a**, XGv347, **b**, XGv289, **c**, XGv282 and **d**, XGv265 are shown. Scala bar in micrographs, 100 nm.



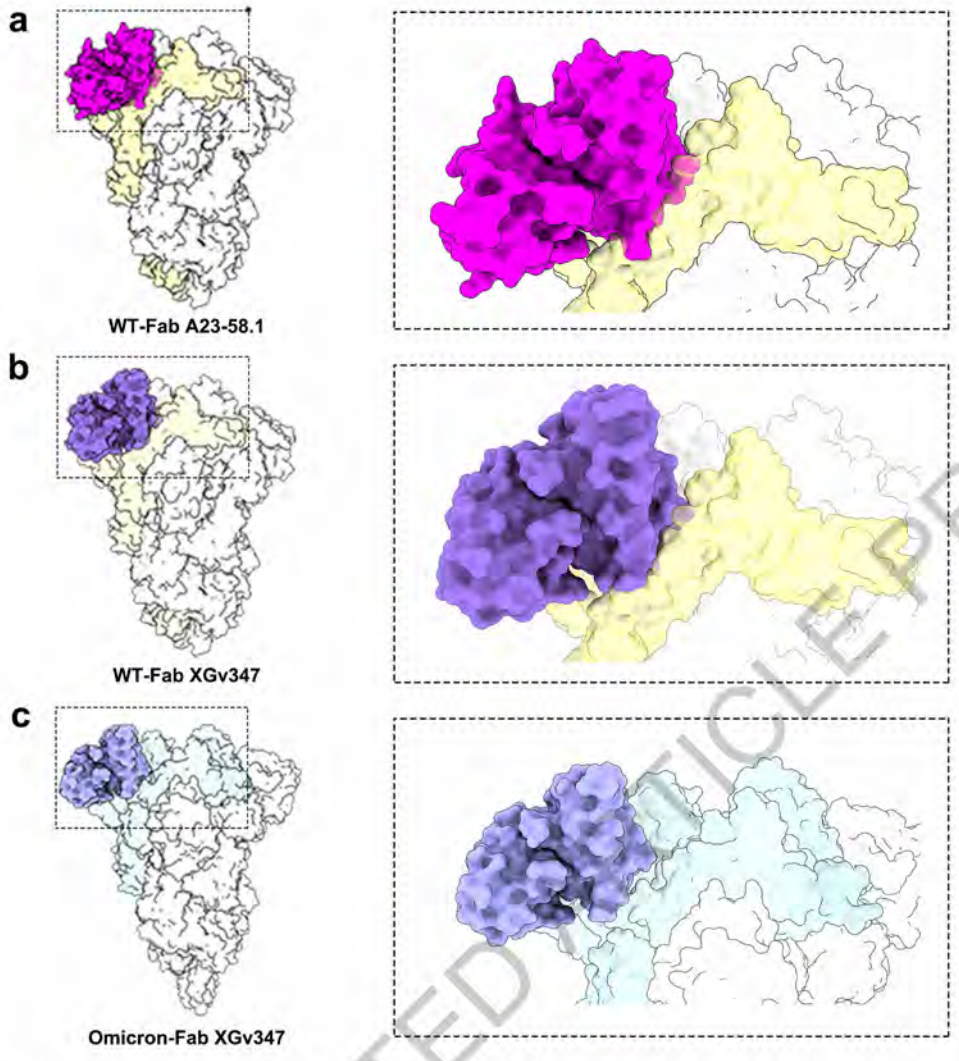
Extended Data Fig. 6 | Resolution estimation of the EM maps. a, The gold-standard FSC curves of overall maps of Omicron S trimer in complex with Fab XGv347, XGv289, XGv282 and XGv265 and local maps of interfaces. **b,** Local resolution assessments of cryo-EM maps using ResMap are shown.



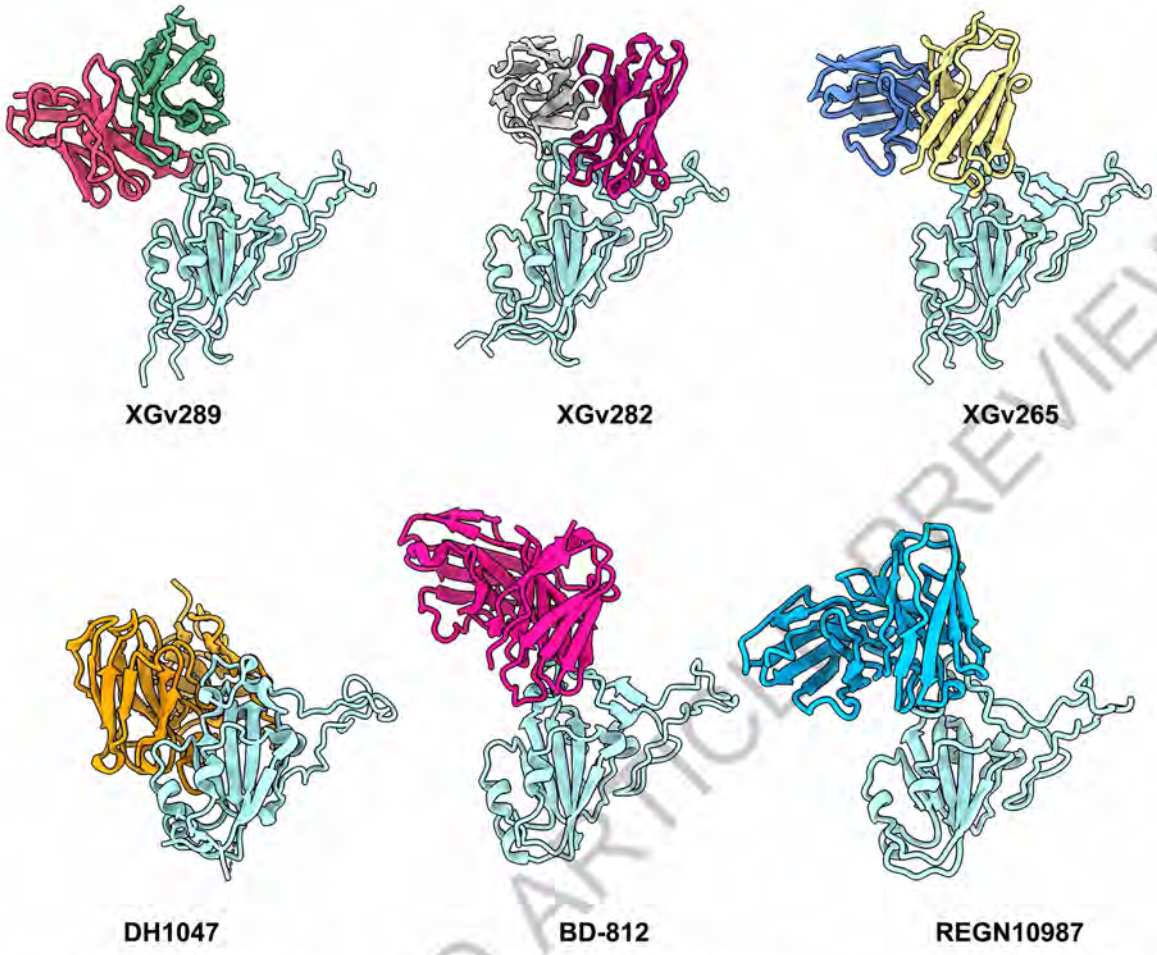
Extended Data Fig. 7 Density maps and atomics models. Cryo-EM density maps of Omicron S trimer in complex with XGv347, XGv289, XGv282 and XGv265 and their interfaces are shown. Color scheme is the same as in Fig. 3**a**. Residues are shown as sticks with oxygen colored in red, nitrogen colored in blue and sulfurs colored in yellow.



Extended Data Fig. 8 Multiple sequence alignment of XGv347, CoV2-2196 and A23-58.1 Multiple sequence alignments of heavy chains and light chains of XGv347, CoV2-2196 and A23-58.1 were performed, respectively. Paratopes of XGv347 binding to Omicron variant RBD are highlighted by green boxes.



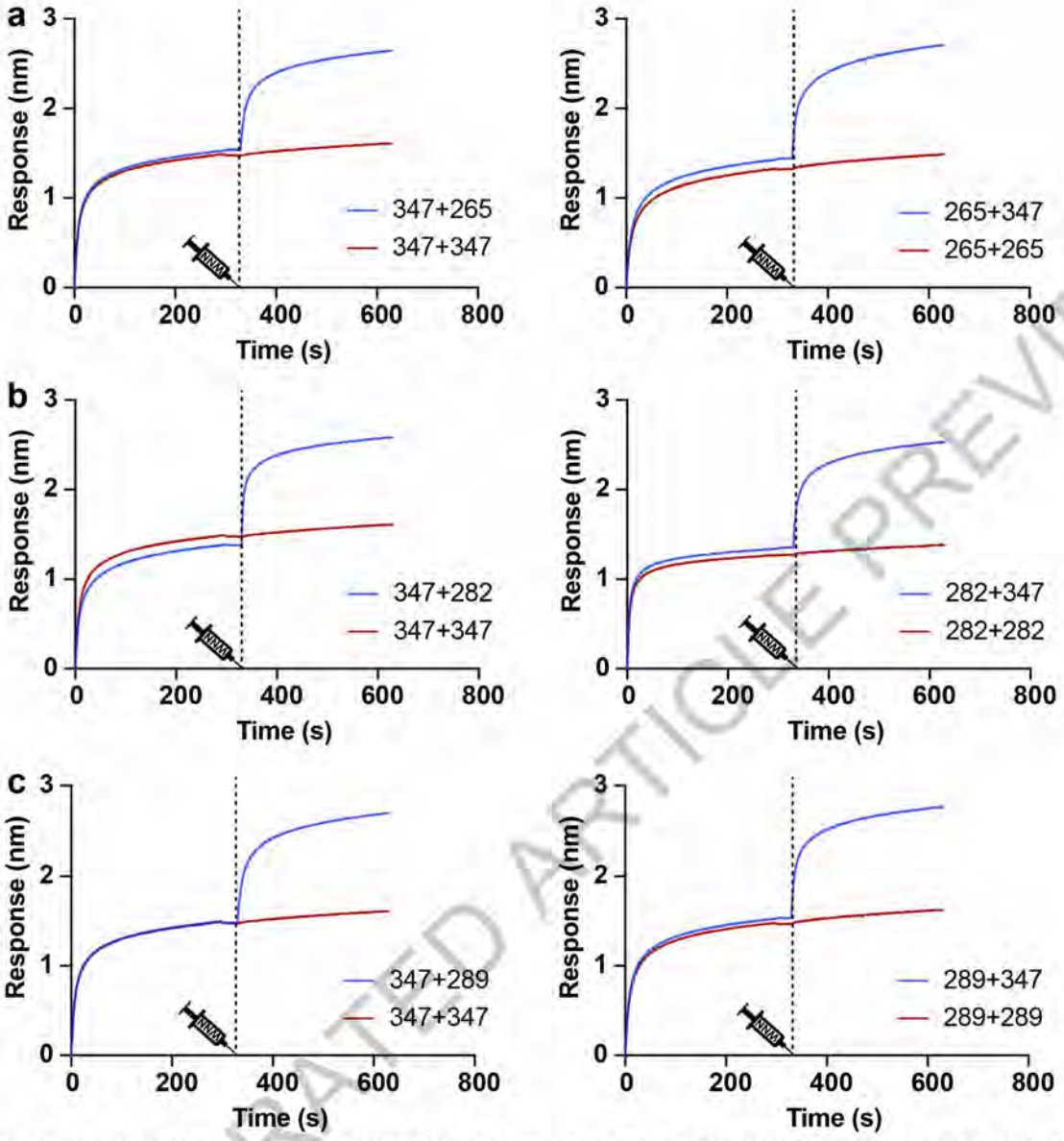
Extended Data Fig. 9 Mechanism of XGv347 binding to 3 closed RBD. a, Superimposition of A23-58.1 onto WT S trimer. **b**, Superimposition of XGv347 onto WT S trimer. **c**, complex of XGv347 and Omicron S trimer. All complexes are in the same orientation with close-ups of Fab-RBD binding modes showing potential clashes.



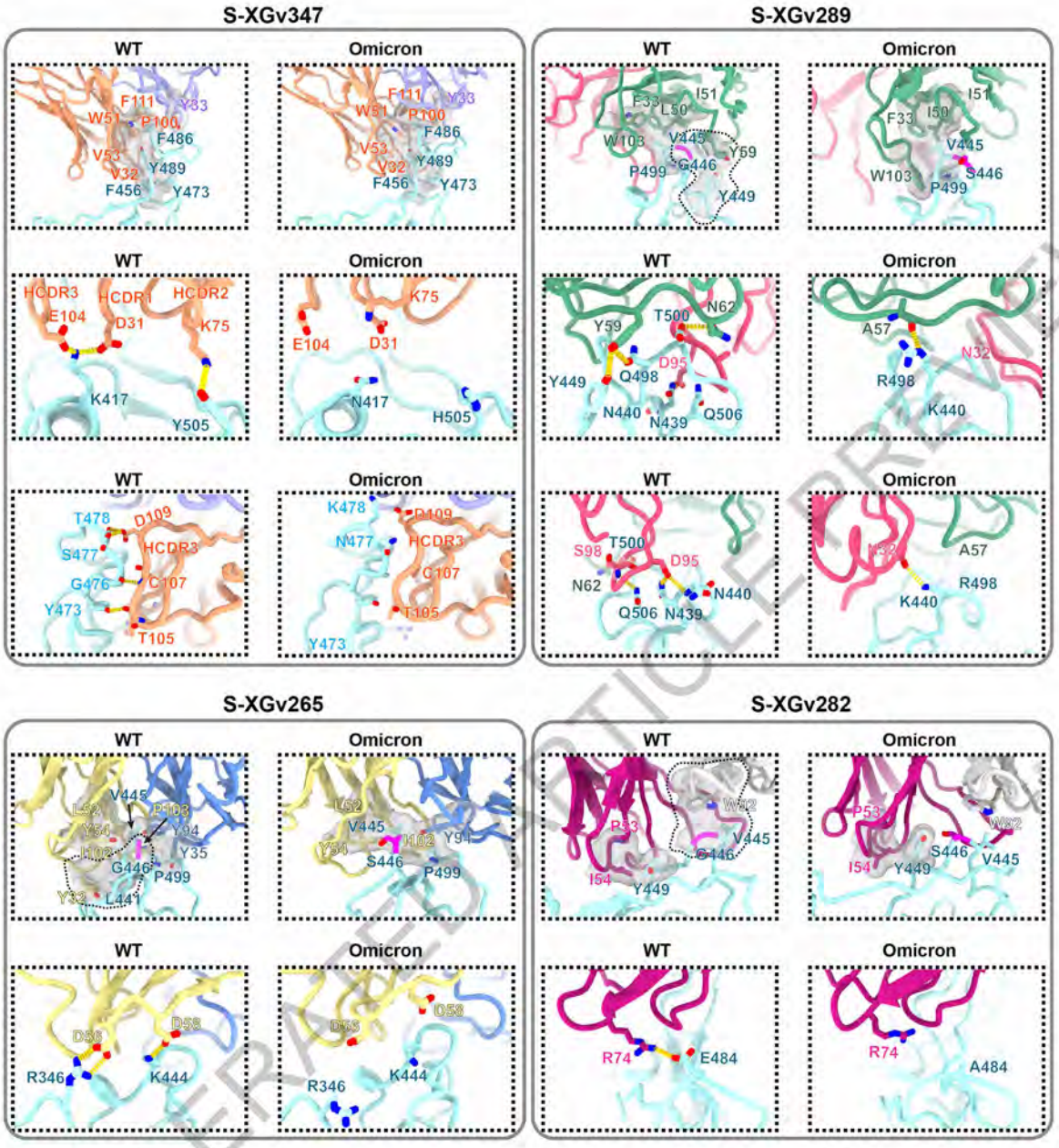
Extended Data Fig. 10 Binding modes of XGv289, 282 and 265. Binding modes of XGv289, XGv282 and XGv265. RBD is colored in light cyan and color scheme of XGv289, XGv282 and XGv265 is the same as in Fig. 3**a**. DH1047, BD-812 and REGN10987 are colored in orange, deep pink and blue, respectively.

XGv289-347 XGv282-347 XGv265-347

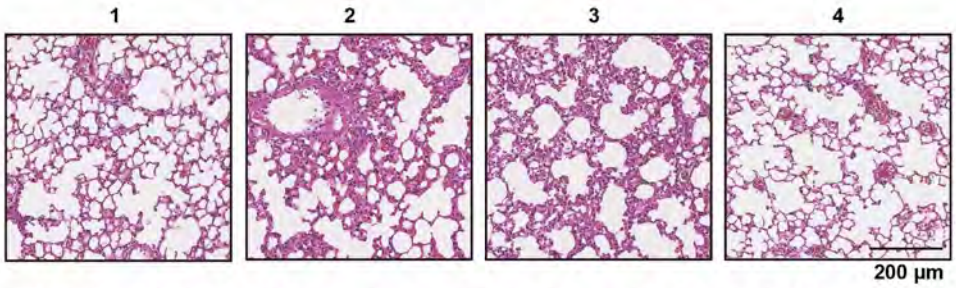
Extended Data Fig. 11 Structural fitting. XGv265, XGv282 and XGv289 are superimposed onto XGv347 and all structure are shown as surface.



Extended Data Fig. 12 BLI assay for XGv347 competing with XGv289, XGv282 and XGv265. Affinity curves of XGv347 to Omicron S protein competing with **a**, XGv265, **b**, XGv282 and **c**, XGv289. In each panel, (left) XGv347 was first injected, followed by the XGv265, XGv282 and XGv289 in **a**, **b** and **c**, respectively. (right) Also, XGv265 in **a**, XGv282 in **b** and XGv289 in **c**, was injected first and competed with the second injection of XGv347. Each curve is a representative of three independent experiments.



Extended Data Fig. 13 Interactions details between antibodies (XGv347, XGv289, XGv282 and XGv265) and SARS-CoV-2 WT (left) and Omicron RBD (right). All the WT structures are predicted with GROMACS. Hydrophobic patches and hydrogen bonds are denoted by surface and dash lines. Color scheme is the same as in Fig.3**a**. For hydrophobic patches of XGv289, XGv282 and XGv265, G446 and S446 are colored in magenta. The dash lines marked out the hydrophobic patches only found in WT RBD.



Extended Data Fig. 14 Histopathological analysis of lung samples from XGv282 treatment group at 5 dpi. Shown here are the H&E staining of lung samples from each of the remaining four mice in XGv282 group. Each micrograph is representative of two separate experiments.

	Omicron S trimer in complex with XGv347 (state 1) EMD-32444 PDB 7WEA	Omicron S trimer in complex with XGv347 (state 2) EMD-32446 PDB 7WEC	Omicron S trimer in complex with XGv347 (state 3) EMD-32445 PDB 7WEB	Omicron S trimer in complex with XGv289 EMD-32443 PDB 7WE9	Omicron S trimer in complex with XGv282 EMD-32441 PDB 7WE7	Omicron S trimer in complex with XGv265 EMD-32442 PDB 7WE8	XGv347-RBD-interface EMD-32447 PDB 7WED	XGv289-RBD-interface EMD-32449 PDB 7WEF	XGv282-RBD-interface EMD-32581 PDB 7WLC	XGv265-RBD-interface EMD-32448 PDB 7WEE
Data collection and processing										
Magnification	22,500	22,500	22,500	22,500	22,500	22,500	22,500	22,500	22,500	22,500
Voltage (kV)	300	300	300	300	300	300	300	300	300	300
Electron exposure (e–/Å²)	60	60	60	60	60	60	60	60	60	60
Defocus range (μm)	-1.5--2.5	-1.5--2.5	-1.5--2.5	-1.5--2.5	-1.5--2.5	-1.5--2.5	-1.5--2.5	-1.5--2.5	-1.5--2.5	-1.5--2.5
Pixel size (Å)	1.07	1.07	1.07	1.07	1.04	1.07	1.07	1.07	1.04	1.07
Symmetry imposed	C1	C3	C1	C1	C1	C1	C1	C1	C1	C1
Initial particle images (no.)	2,320,416	2,320,416	2,320,416	2,332,045	756,508	1,302,103	2,320,416	2,332,045	756,508	1,302,103
Final particles images (no.)	269,947	85,822	105,455	401,170	119,800	138,359	527,413	401,170	119,800	138,359
Map resolution (Å)	3.3	3.3	3.7	3.6	3.8	3.5	3.5	3.8	4.0	3.9
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
Map resolution range (Å)	3.3-60	3.3-60	3.7-60	3.6-60	3.8-60	3.5-60	3.5-60	3.8-60	4.0-60	3.9-60
Refinement										
Initial model used (PDB code)	7CWL	7CWL	7CWL	7CWL	7CWL	7CWL	7CWL	7CWL	7CWL	7CWL
Model resolution (Å)	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
Model resolution range (Å)	3.8-60	3.8-60	3.8-60	3.8-60	3.8-60	3.8-60	3.8-60	3.8-60	3.8-60	3.8-60
Map sharpening *B* factor (Å²)	135.7	137.1	134.8	166.0	161.3	165.3	250.0	216.9	200.0	197.2
Model composition										
Non-hydrogen atoms	30,530	32,320	30,488	32,034	31,881	28,730	3,358	3,328	3,307	3,350
Protein residues	3,754	3,984	3,754	3,987	3,981	3,522	431	432	430	429
Ligands	78	81	75	67	61	75	0	0	0	0
B factors (Å²)										
Protein	109.94	107.91	145.86	120.89	134.24	190.94	54.12	63.36	111.21	59.42
Ligand	127.05	136.95	164.34	142.43	151.94	214.20	-	-	-	-
R.m.s. deviations										
Bond lengths (Å)	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.006	0.005
Bond angles (°)	0.590	0.594	0.533	0.563	0.570	0.555	0.700	0.624	1.223	0.720
Validation										
MolProbity score	1.90	1.87	1.87	1.99	1.95	2.00	1.88	1.93	1.81	1.76
Clashscore	9.71	8.88	9.96	10.36	12.49	11.20	6.98	8.46	6.34	8.20
Poor rotamers (%)	0.00	0.00	0.00	0.03	0.06	0.03	0.00	0.00	0.00	0.00
Ramachandran plot										
Favored (%)	94.17	94.07	94.98	92.73	95.08	93.35	91.76	92.49	92.69	95.51
Allowed (%)	5.75	5.90	4.99	7.19	4.92	6.62	8.24	7.51	7.31	4.49
Disallowed (%)	0.08	0.03	0.03	0.08	0.00	0.03	0.00	0.00	0.00	0.00

Extended Data Table. 1 Statistics for cryo-EM data collection, refinement, and validation.

Complex	Omicron RBD	Heavy chain				Light chain		
Omicron S-trimer in complex with XGv265	ARG 346	Y32						
	ASN 439					Y35		
	ASP 442	Y32						
	SER 443	I102						
	LYS 444	Y54	D56	D58	W55			
	VAL 445	Y54	R60	L52		T99		
	SER 446	R60						
	GLY 447	R60						
	PRO 499					Y35	Y94	
	ARG 509	Y32						
Omicron S-trimer in complex with XGv289	PHE 374					N32		
	PHE 375					Y33		
	ASN 439	S101				D95		
	LYS 440	S102	S101			Y33		
	SER 443	S101						
	VAL 445	A57						
	SER 446	A57	S58	G56				
	PRO 499	S101						
	THR 500	N62	A60	Q61		G99	S98	
	GLY 502	N62				L97	S98	
	VAL 503					D95	S96	L97
Omicron S-trimer in complex with XGv347	LEU 455	D31						
	PHE 456	D31	V32					
	TYR 473	T105						
	ALA 475	S106						
	GLY 476	C107						
	LYS 478	D109						
	GLY 485	W51						
	PHE 486	P100	S108	D109	F111	Y33		
	ASN 487	S108						
	TYR 489	V32	S34	V53				
	ARG 493	G55	T56					
Omicron S-trimer in complex with XGv282	K440	F103						
	S443	F103						
	K444	G102						
	V445	G102	F103	D104		W92		
	S446	G102						
	Y449	S31	R50	I52	I54			
	L452	I54						
	F490	R74						
	R498					W92		

Extended Data Table. 2 List of interacting residues between Fabs and Omicron SARS-CoV-2 S trimer (d < 4 Å).

Complex	KD (nM)	ΔG (kcal/mol)	ΔΔG (kcal/mol)	No. (residue$_{TOTAL}$)	No. (residue$_{RBD}$)	No. (residue$_{Fab}$)	No. (HB or SB)	No. (nonpolar residue$_{RBD}$)	No. (nonpolar residue$_{Fab}$)
WT RBD in complex with XGv265	1.475	-3.99	-0.96	21	10	11	14	4	7
Omicron RBD in complex with XGv265	28.52	-3.03		21	10	11	9	3	7
WT RBD in complex with XGv282	0.8612	-3.79	-1.82	28	15	13	13	7	8
Omicron RBD in complex with XGv282	4.096	-1.97		19	8	11	7	3	8
WT RBD in complex with XGv289	1.287	-5.94	-0.79	21	9	12	16	4	6
Omicron RBD in complex with XGv289	14.17	-5.15		26	11	15	12	3	4
WT RBD in complex with XGv347	0.1518	-5.42	-0.14	23	10	13	15	4	5
Omicron RBD in complex with XGv347	6.812	-5.28		26	11	15	9	4	5

Extended Data Table. 3 Statistics for molecular dynamics.

HB, hydrogen bond; SB, salt bridge.

nature portfolio

Corresponding author(s): Xiangxi Wang

Last updated by author(s): Jan 12, 2022

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Serial EM3.8
Data analysis	GROMACS2021,RELION 3.0, cryoSPARC 3.3.1, Chimera 1,15, Chimerax 1.1, coot0.9.4, Phenix 1.19 and GraphPad Prism 9.2.0 were used for data analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data availabilityThe atomic coordinates of XGv347 in complex with S trimer (state I), XGv347 in complex with S trimer (state II), XGv347 in complex with S trimer (state III), XGv347-S have been submitted to the Protein Data Bank with accession numbers: 7WEA, 7WEC and 7WEB, respectively. Futhermore, the atomic coordinates of XGv265, XGv282 and XGv289 have been deposited in the protein data bank under accession code 7WE8, 7WE7 and 7WE9, respectively. Cryo-EM density maps in this study have been deposited at the Electron Microscopy Data Bank with accession codes EMD-32444 (state1), EMD-32446 (state2) and EMD-32445 (state3), EMD-32441 (XGv282), EMD-32442 (XGv265), and EMD-32443 (XGv289). To reveal structural details of Fab binding mechanism, the local optimized method are used to optimized data progress and the related atomic models and EM density maps of optimized reconstructions of Fab interaction

Interface has been deposited under accession code 7WEE (XGv265), 7WED (XGv347), 7WLC (XGv282), 7WEF (XGv289), EMD-32447 (XGv347), EMD-32448 (XGv265), EMD-32581(XGv282), EMD-32449 (XGv289), respectively.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample size. We obtained 120 human serums, all the vaccine sera were collected from volunteers who received two doses or three doses of the WHO-approved inactivated SARS-CoV-2 vaccine (CorovaVac, Sinovac, China). For the animal study, 37 BALB/c mice and 10 K18-hACE2 mice were used for protection experiments.
Data exclusions	No data excluded.
Replication	All experiments were performed and verified in multiple replicates as indicated in their methods/figure legends.
Randomization	All mice were divided into the given groups (7 for Beta strain challenge and 2 for Omicron strain challenge) randomly.
Blinding	Volunteers received vaccinations open-label. The investigators were not blinded to allocation during experiments and outcome assessment. Data collection and analysis were performed by different people, the sample classification were replaced by simple marks during data analysis

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	XGv347,XGv286,XGv051,XGv303,XGv264,XGv293,XGv052,XGv337,XGv338,XGv261,XGv302,XGv289,XGv291,XGv042,XGv297,XGv288, XGv055,XGv295,XGv345,XGv074,XGv016,XGv292,XGv290,XGv304,XGv031,XGv282,XGv053,XGv387,XGv296,XGv253,XGv287,XGv294, XGv050,XGv017,XGv177,XGv026,XGv265,XGv420,XGv402,XGv285,XGv266, VIR-7831, DXP-604, AZD8895, REGN10987, LY-CoV016
Validation	All of the XGv series SARS-CoV-2 spike antigen-specific monoclonal antibodies have been validated for use in ELISA, BLI and neutralizing SARS-CoV-2 pseudovirus/authentic virus first time in this study. S309, VIR-7831, DXP-604, AZD8895, REGN10987, LY-CoV016 have been validated in previous publications cited in this paper. Specifically,VIR-7831 was tested in Pinto, D et al 2020, Nature;DXP-604 was tested in Shuo, D. et al 2020, Cell; AZD8895 was tested in Jinhui,D. et al 2021,Nat Microbiol; REGN10987 was tested in Johanna Hansen et al 2020, Science;LY-CoV016 was tested in Shi, R. et al 2020, Nature.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T cells (ATCC, cat. no. CRL-3216), Huh-7 cells (Japanese Collection of Research Bioresources [JCRB], cat. no. 0403),HEK293F cells (Thermo Fisher, cat. no. 11625019)
Authentication	The authentication of cells have been confirmed using STR method

nature portfolio | reporting summary

March 2021

2

Mycoplasma contamination	These cell lines tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	None

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Groups of BALB/c and K18-hACE2 mice were used. All mice were group-housed conventionally on a 12-h light/dark cycle for 3 days before any experiments,the environmental conditions were maintained thermostatically between 18°C-23°C with 40%-60% humidity.
Wild animals	No wild animals were used in the study
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	All procedures associated with SARS-CoV-2 live virus were approved by the Animal experiment Committee Laboratory Animal Center, Beijing Institute of Microbiology and Epidemiology with an approval number of IACUC-IME-2021-022 and performed in Biosafety Level 3 (BSL-3) laboratories in strict accordance with the recommendations in the Guide for Care and Use of Laboratory Animals.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Plasma samples were obtained from volunteers who received two doses or three doses of the WHO-approved inactivated SARS-COV-2 vaccine (CorovaVac, Sinovac, China).Median age of participants was 37 years. 44% of volunteers were males and 56% were females.
Recruitment	All the volunteers were recruited by Sinovac, Inc. None of the volunteers had a history of prior SARS-CoV-2 infection and none reported serious adverse events after vaccination.
Ethics oversight	The procedures about human participants were approved by the Ethics Committee (seal) of Beijing Youan Hospital, Capital Medical University with an approval number of LL-2021-042-K. All participants provided written informed consent.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

nature portfolio | reporting summary
March 2021

2.5. Dose de reforço da CoronaVac pode neutralizar variantes de preocupação, indica estudo

Dados de uma pesquisa divulgada em carta ao editor na revista Emerging Microbes & Infections mostraram que a terceira dose da CoronaVac protege não só contra a cepa original do SARS-CoV-2, mas também contra as variantes alfa, beta e delta. Além disso, a memória das células T pode ser despertada rapidamente após a dose de reforço, caso o indivíduo entre em contato com o vírus. Publicado em novembro de 2021, o estudo foi conduzido por cientistas chineses do Instituto de Biomedicina da Academia Chinesa de Ciências Médicas.

Os pesquisadores avaliaram a capacidade de proteção contra as variantes alfa, beta e delta em amostras de sangue de 53 pacientes vacinados com a CoronaVac e de 12 modelos animais, decorridos 14 dias após a dose de reforço – administrada oito meses depois da segunda dose.

A taxa de soroconversão excedeu 90% e os anticorpos presentes nos soros foram capazes de neutralizar as variantes, apesar de terem diminuído até 5,6 vezes contra essas cepas em relação à original.

Em etapa anterior da pesquisa, seis dos 53 voluntários tiveram amostras coletadas aos cinco, sete e 14 dias após a dose de reforço, para a detecção de anticorpos IgG, anticorpos neutralizantes e resposta de células T de memória contra a cepa original do SARS-CoV-2.

Os anticorpos IgG e os anticorpos neutralizantes aumentaram gradualmente após cinco dias e a taxa de soroconversão atingiu 100% em 14 dias. A resposta de células T também se mostrou rápida.

"Os achados indicam que, embora os anticorpos neutralizantes diminuam com o tempo após duas doses, a resposta de anticorpos pode ser despertada rapidamente com a terceira dose e a memória imunológica das células T ainda está ativa", informam os autores.

Os cientistas acrescentam que é essencial continuar analisando a persistência da imunidade e a efetividade da dose de reforço das vacinas, conduzindo ensaios clínicos de longo prazo.

Publicado em: 16/11/2021

Emerging Microbes & Infections

Taylor & Francis

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/temi20

Antibody response elicited by a third boost dose of inactivated SARS-CoV-2 vaccine can neutralize SARS-CoV-2 variants of concern

Lei Yue, Jian Zhou, Yanan Zhou, Xiaolei Yang, Tianhong Xie, Mengli Yang, Hongling Zhao, Yuan Zhao, Ting Yang, Hua Li, Hong Xiang, Jie Wang, Shuaiyao Lu, Hongqi Liu, Hong Zhao, Xingchen Wei, Yuhao Zhang & Zhongping Xie

To cite this article: Lei Yue, Jian Zhou, Yanan Zhou, Xiaolei Yang, Tianhong Xie, Mengli Yang, Hongling Zhao, Yuan Zhao, Ting Yang, Hua Li, Hong Xiang, Jie Wang, Shuaiyao Lu, Hongqi Liu, Hong Zhao, Xingchen Wei, Yuhao Zhang & Zhongping Xie (2021) Antibody response elicited by a third boost dose of inactivated SARS-CoV-2 vaccine can neutralize SARS-CoV-2 variants of concern, Emerging Microbes & Infections, 10:1, 2125-2127, DOI: 10.1080/22221751.2021.1996210

To link to this article: https://doi.org/10.1080/22221751.2021.1996210

Published online: 16 Nov 2021.

Emerging Microbes & Infections
2021, VOL. 10
https://doi.org/10.1080/22221751.2021.1996210

LETTER

OPEN ACCESS Check for updates

Antibody response elicited by a third boost dose of inactivated SARS-CoV-2 vaccine can neutralize SARS-CoV-2 variants of concern

Lei Yue, Jian Zhou, Yanan Zhou, Xiaolei Yang, Tianhong Xie, Mengli Yang, Hongling Zhao, Yuan Zhao, Ting Yang, Hua Li, Hong Xiang, Jie Wang, Shuaiyao Lu, Hongqi Liu, Hong Zhao, Xingchen Wei, Yuhao Zhang and Zhongping Xie

The Institute of Medical Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, Kunming, People's Republic of China

ARTICLE HISTORY Received 29 August 2021; Revised 14 October 2021; Accepted 16 October 2021

Dear editor

Since the initial outbreak in late 2019, coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, has evolved into a global pandemic [1,2]. Inactivated vaccines [3], mRNA vaccines [4], and adenovirus vector vaccines [5] have been developed based on different platforms. Several vaccines have obtained emergency use authorization from the World Health Organization. Recently, the U.S. Food and Drug Administration approved the first COVID-19 vaccine (Pfizer-BioNTech COVID-19 Vaccine) [6]. Mass vaccination has played an important role in the effective control of COVID-19 epidemic worldwide [7].

Inactivated SARS-CoV-2 vaccines have been mainly developed by companies in developing countries, and clinical trials showed good safety profiles and protect against COVID-19 [3,8,9]. Inactivated vaccines have been approved by dozens of countries and jurisdictions [10]. Current research shows that 6 months after two doses of inactivated vaccine, the neutralizing antibody wanes significantly, although the immune memory is not disappeared [11]. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection [12]. Therefore, the necessity of the third booster dose is constant concerned. In addition, facing the constantly emerging variants of concern, it is still uncertain whether a booster dose of inactivated vaccine can evoke immune memory quickly to provide important protection.

In this study, 53 volunteers, who joined in the development and production of inactivated vaccines (with informed consent), received two doses (at 0 and 28 days) of inactivated COVID-19 vaccines in 2020. Due to the need to further explore COVID-19 vaccines, they received a third dose 8 months after the second dose recently. At 0, 5, 7, and 14 days after the third dose, blood was collected from 6 volunteers for detection of anti-S IgG antibody (Figure 1A), neutralizing antibody titre (Figure 1B) and specific IFN-γ-secreting T-cell response (Figure 1C). We found that both the anti-S antibody and neutralizing antibody against the original strain (GD108#) gradually increased after 5 days, and the positive conversion rate of antibodies reached 100% at 14 days. Interestingly, the memory of IFN-γ-T cells against S, N, M, O antigens of SARS-CoV-2 can be quickly awakened after the third dose. These results indicate that although the neutralizing antibodies gradually decrease after two doses of inactivated vaccines, the antibody response could be awakened quickly and the T-cell immune memory is still active.

To address the question that whether a third booster dose could provide protection to variants of concern. Here, we assessed cross-protection capacity against alpha, beta and delta variants on 53 human sera and 12 monkey sera of 14 days after the third booster dose. It is encouraging that the neutralizing antibody can neutralize recently emerged SARS-CoV-2 variants, and the antibody-positive conversion rate exceeds 90%, even if the human neutralizing antibodies titre decreased approximately 1.9, 5.4, 4.2 times against alpha, beta and delta variants, respectively, and the monkey neutralizing antibodies decreased approximately 3.0, 5.6, 4.6 times (Figure 1D–G).

Our studies provided evidence for the efficacy of a third booster dose of inactivated SARS-CoV-2 vaccine against variants of concern. However, it is necessary to evaluate its effectiveness in the real world in the future. A recent real-world study conducted in Guangzhou

CONTACT Zhongping Xie xzp218@hotmail.com The Institute of Medical Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, Kunming, Yunnan 650118, People's Republic of China
Lei Yue and Jian Zhou contribute equally to this work.

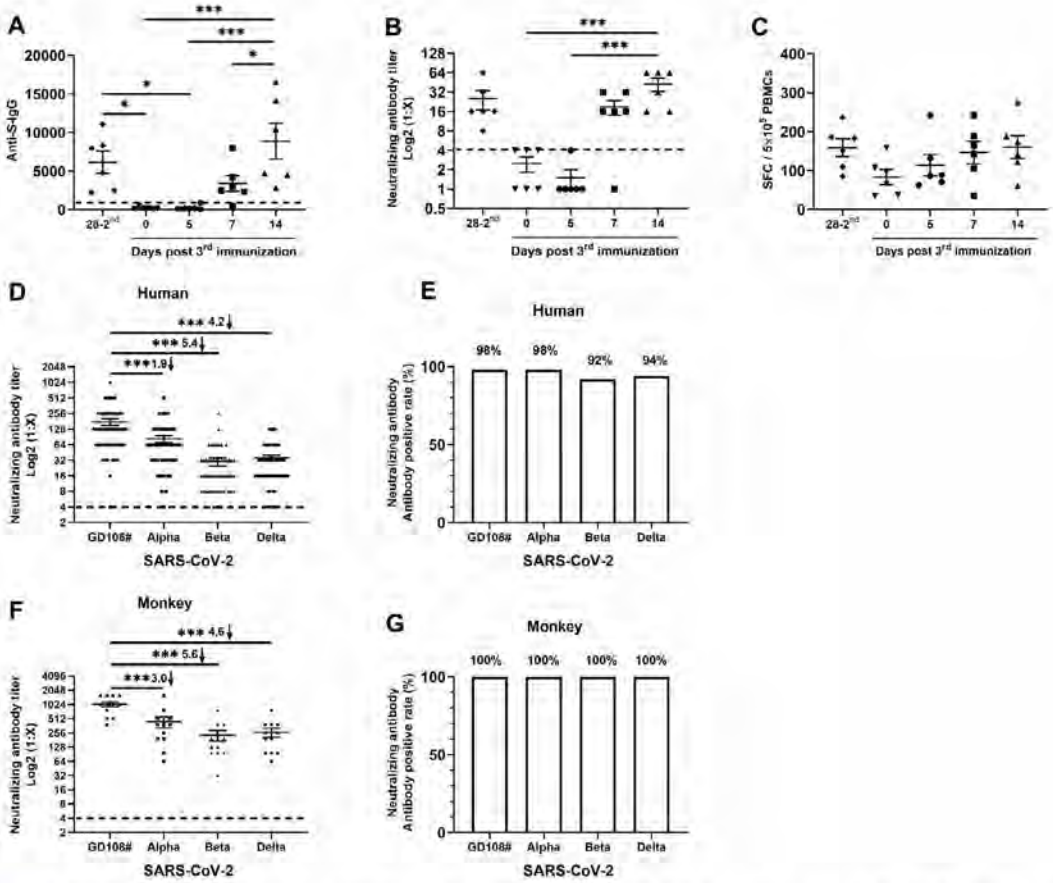


Figure 1. Antibody response elicited by a third boost dose of inactivated SARS-CoV-2 vaccine can neutralize SARS-CoV-2 variants of concern. (A–C) Enzyme-linked immunosorbent assay (ELISA) antibody against S protein, neutralizing antibody against original strain (GD108#), and the IFN-γ-specific T-cell responses against the S, N, M, and O antigens induced by a third booster dose of inactivated SARS-CoV-2 vaccine ($n = 6$). (D and E) Human neutralizing antibodies and positive rate against original strain (GD108#) and variants (alpha, beta, delta) induced by the inactivated SARS-CoV-2 vaccine 14 days after a third booster dose ($n = 53$). (F, G) Monkey neutralizing antibodies and positive rate against original strain (GD108#) and variants induced by the inactivated SARS-CoV-2 vaccine 14 days after a third booster dose ($n = 12$). The neutralizing antibody-positive judgment threshold is marked with a dotted line. (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

(China) showed that the protection rate of two doses inactivated vaccine against delta variant infection exceeded 50% [13]. In addition, vaccination with CoronaVac was associated with a reduction in symptomatic Covid-19, hospital admissions, and deaths in adults aged ≥70 years in a setting with extensive transmission of the gamma variant in Brazil [10]. Given that the neutralizing antibody of 1 month after a third booster dose is significantly higher than that of a two-dose procedure [14], we believe that a three-dose procedure may be more effective against variants. Continuously observing the persistence of the protection provided by vaccines in real cases and the effectiveness of a third booster dose, conducting long-term clinical trials, and obtaining post clinical data are essential tasks.

In short, vaccination with inactivated vaccines is still an effective way to fight against the SARS-CoV-2 and variants epidemic.

Data availability

The results supporting the findings in this study are available upon request from the corresponding authors.

Author contributions

Conceptualization: L.Y. and Z.X.; methodology: L.Y., J.Z., and Y.Z.; investigation: L.Y., J.Z., Y.Z., X.Y., T.X., M.Y., H.Z., Y.Z., T.Y., H.L., H.X., and J.W.; resources: S.L. and H.L; data curation: L.Y., J.Z., H.Z., X.W., Y.Z., and Z.X.; writing—original draft: L.Y.; writing—review & editing: L.Y., J.Z., and Z.X.; supervision: Z.X.; funding acquisition: L.Y. and Z.X.

Acknowledgements

We thank Dr. Zhuoming Liu (Washington University School of Medicine, Saint Louis, USA) for input during the writing of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by National Natural Science Foundation of China: [Grant Number 81800012]; Yunnnan Provincial Science and Technology Department: [Grant Number 202105AC160025]; Chinese Academy of Medical Sciences: [Grant Number 2020-I2M-CoV19-012]; Health Commission of Yunnan Province: [Grant Number H-2018061]; Kunming Science and Technology Bureau: [Grant Number 2020-1- H-021].

ORCID

Lei Yue http://orcid.org/0000-0001-5201-4373
Zhongping Xie http://orcid.org/0000-0003-2687-4438

References

[1] Zhu N, Zhang D, Wang W, et al. A novel Coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Feb 20;382(8):727–733.
[2] Holshue ML, DeBolt C, Lindquist S, et al. First case of 2019 novel coronavirus in the United States. N Engl J Med. 2020 Mar 5;382(10):929–936.
[3] Al Kaabi N, Zhang Y, Xia S, et al. Effect of 2 inactivated SARS-CoV-2 vaccines on symptomatic COVID-19 infection in adults: a randomized clinical trial. JAMA. 2021 Jul 6;326(1):35–45.
[4] Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021 Feb 4;384(5):403–416.
[5] Ramasamy MN, Minassian AM, Ewer KJ, et al. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. Lancet. 2021 Dec 19;396(10267):1979–1993.
[6] FDA Approves First COVID-19 Vaccine 2021. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-first-covid-19-vaccine
[7] Hasan T, Beardsley J, Marais BJ, et al. The implementation of mass-vaccination against SARS-CoV-2: a systematic review of existing strategies and guidelines. Vaccines (Basel). 2021 Apr 1;9(4):326.
[8] Ella R, Vadrevu KM, Jogdand H, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: a double-blind, randomised, phase 1 trial. Lancet Infect Dis. 2021 May;21(5):637–646.
[9] Jara A, Undurraga EA, Gonzalez C, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. N Engl J Med. 2021 Sep 2;385(10):875–884.
[10] Ranzani OT, Hitchings MDT, Dorion M, et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study. Br Med J. 2021 Aug 20;374(n2015).
[11] Liao Y, Zhang Y, Zhao H, et al. Intensified antibody response elicited by boost suggests immune memory in individuals administered two doses of SARS-CoV-2 inactivated vaccine. Emerg Microbes Infect. 2021 Dec;10(1):1112–1115.
[12] Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med. 2021 Jul;27(7):1205–1211.
[13] Li XN, Huang Y, Wang W, et al. Efficacy of inactivated SARS-CoV-2 vaccines against the delta variant infection in Guangzhou: A test-negative case-control real-world study. Emerg Microbes Infect. 2021 Dec;10(1):1751–1759.
[14] Yue L, Xie T, Yang T, et al. A third booster dose may be necessary to mitigate neutralizing antibody fading after inoculation with two doses of an inactivated SARS-CoV-2 vaccine. J Med Virol. 2021 Sep 13.

CoronaVac

O que a ciência comprova

2.6. CoronaVac tem eficácia superior a 75% contra variantes alfa, gama e delta; apenas 2% dos chilenos vacinados na fase 3 desenvolveram Covid-19

Duas pesquisas publicadas por cientistas chilenos dão provas de que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, é eficiente no combate à Covid-19 e eficaz contra as novas variantes do SARS-CoV-2. No primeiro estudo, os indicadores de anticorpos neutralizantes gerados pelo imunizante foram acima de 97% contra a cepa original do vírus, acima de 80% contra as variantes alfa e gama e acima de 75% contra a variante delta. No segundo estudo, a eficácia da CoronaVac para evitar o desenvolvimento de casos de Covid-19 foi superior a 90% em um grupo de mais de duas mil pessoas.

Ambas as pesquisas são de autoria de cientistas da Pontifícia Universidade Católica do Chile, do Instituto de Saúde Pública do Chile e da Universidade do Chile e foram publicadas na revista científica Frontiers of Immunology. A importância dos estudos se deve ao fato de que a vacinação no país andino foi feita preponderantemente com a CoronaVac, com 70% das pessoas recebendo o imunizante do Butantan.

Eficácia da CoronaVac contra as variantes do SARS-CoV-2

De acordo com o estudo Recognition of variants of concern by antibodies and T cells induced by a SARS-CoV-2 inactivated vaccine, a CoronaVac promoveu a secreção de anticorpos capazes de bloquear o domínio receptor-obrigatório (RBD, do inglês receptor-binding domain, partes específicas do coronavírus que lhe permitem invadir e infectar células humanas) de todas as variantes de preocupação do SARS-CoV-2. As taxas de soropositividade de anticorpos neutralizantes registradas foram acima de 97% para a cepa original, de mais de 80% para as variantes alfa e gama, de mais de 75% para a variante delta e de mais de 60% para a variante beta.

Para fazer essa análise, os pesquisadores avaliaram os voluntários inscritos no ensaio clínico de fase 3 que foram imunizados com duas doses de CoronaVac no Chile. Após a administração da segunda dose,

foram coletadas amostras de soro para medir a capacidade de neutralização de anticorpos contra as variantes de preocupação. "É importante ressaltar que, após a infecção por SARS-CoV-2, a capacidade de bloqueio de anticorpos de voluntários vacinados aumentou para todas as variantes testadas", ressaltaram os cientistas. Segundo eles, a imunização com CoronaVac em qualquer esquema estimula respostas celulares contra todas as variantes de preocupação e contribui para neutralizar a infecção causada pelo vírus.

Entre 2.263 chilenos vacinados com CoronaVac, apenas 45 desenvolveram Covid-19

Já o estudo Immune Profile and Clinical Outcome of Breakthrough Cases After Vaccination with an inactivated SARS-CoV-2 Vaccine avaliou a segurança, a imunogenicidade e a eficácia da CoronaVac para evitar casos graves de Covid-19. Dos 2.263 indivíduos totalmente vacinados no final de junho de 2021, apenas 45 (ou seja, 1,99%) apresentaram sintomas de infecção sintomática decorridos 14 dias ou mais da segunda dose.

Destes 45, 43 desenvolveram quadros leves. As exceções foram dois casos de homens com mais de 60 anos. O primeiro deles, um homem de 62 anos com duas comorbidades (hipotireoidismo e obesidade), desenvolveu um quadro moderado e necessitou de oxigenação suplementar. O segundo, um homem de 69 anos com quatro comorbidades (obesidade, hipertensão, aorta bicúspide e fibrilação atrial), evoluiu para um quadro de maior gravidade e precisou de ventilação mecânica. Ambos se restabeleceram e passam bem.

Os pesquisadores salientaram que a vacinação com CoronaVac é eficaz. "Os casos da doença foram em sua maioria leves e não necessariamente se correlacionaram à falta de imunidade induzida pela vacina, sugerindo que outros fatores, a serem definidos em estudos futuros, poderiam levar à infecção sintomática após a vacinação com CoronaVac."

Publicados em: 9/11/2021 e 29/09/2021

frontiers in Immunology

ORIGINAL RESEARCH
published: 09 November 2021
doi: 10.3389/fimmu.2021.747830

Recognition of Variants of Concern by Antibodies and T Cells Induced by a SARS-CoV-2 Inactivated Vaccine

Felipe Melo-González[1,2†], Jorge A. Soto[1,2†], Liliana A. González[1,2†], Jorge Fernández[3†], Luisa F. Duarte[1,2†], Bárbara M. Schultz[1,2†], Nicolás M. S. Gálvez[1,2†], Gaspar A. Pacheco[1,2], Mariana Ríos[1,2], Yaneisi Vázquez[1,2], Daniela Rivera-Pérez[1,2], Daniela Moreno-Tapia[1,2], Carolina Iturriaga[4], Omar P. Vallejos[1,2], Roslye V. Berríos-Rojas[1,2], Guillermo Hoppe-Elsholz[1,2], Marcela Urzúa[4], Nicole Bruneau[3], Rodrigo A. Fasce[3], Judith Mora[3], Alba Grifoni[5], Alessandro Sette[5,6], Daniela Weiskopf[5], Gang Zeng[7], Weining Meng[7], José V. González-Aramundiz[8], Pablo A. González[1,2], Katia Abarca[1,4], Eugenio Ramírez[3], Alexis M. Kalergis[1,2,9*] and Susan M. Bueno[1,2*]*

[1] Millennium Institute on Immunology and Immunotherapy, Pontificia Universidad Católica de Chile, Santiago, Chile, [2] Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile, [3] Departamento de Laboratorio Biomédico, Instituto de Salud Pública de Chile, Santiago, Chile, [4] Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, División de Pediatría, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile, [5] Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology (LJI), La Jolla, CA, United States, [6] Department of Medicine, Division of Infectious Diseases and Global Public Health, University of California, San Diego (UCSD), La Jolla, CA, United States, [7] Sinovac Biotech, Beijing, China, [8] Departamento de Farmacia, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile, [9] Departamento de Endocrinología, Facultad de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus responsible of the current pandemic ongoing all around the world. Since its discovery in 2019, several circulating variants have emerged and some of them are associated with increased infections and death rate. Despite the genetic differences among these variants, vaccines approved for human use have shown a good immunogenic and protective response against them. In Chile, over 70% of the vaccinated population is immunized with CoronaVac, an inactivated SARS-CoV-2 vaccine. The immune response elicited by this vaccine has been described against the first SARS-CoV-2 strain isolated from Wuhan, China and the D614G strain (lineage B). To date, four SARS-CoV-2 variants of concern described have circulated worldwide. Here, we describe the neutralizing capacities of antibodies secreted by volunteers in the Chilean population immunized with CoronaVac against variants of concern Alpha (B.1.1.7), Beta (B.1.351) Gamma (P.1) and Delta (B.617.2).

Methods: Volunteers enrolled in a phase 3 clinical trial were vaccinated with two doses of CoronaVac in 0-14 or 0-28 immunization schedules. Sera samples were used to evaluate the capacity of antibodies induced by the vaccine to block the binding between Receptor Binding Domain (RBD) from variants of concern and the human ACE2 receptor by an in-house ELISA. Further, conventional microneutralization assays were used to test neutralization of SARS-CoV-2 infection. Moreover, interferon-γ-secreting T cells

OPEN ACCESS

Edited by:
Xuguang (Sean) Li,
Health Canada, Canada

Reviewed by:
Sathya Narayanan Thulasi Raman,
Health Canada, Canada
Robert J. Visalli,
Mercer University, United States

***Correspondence:**
Eugenio Ramírez
eramirez@ispch.cl
Alexis M. Kalergis
akalergis@bio.puc.cl
Susan M. Bueno
sbueno@bio.puc.cl

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Vaccines and Molecular Therapeutics, a section of the journal Frontiers in Immunology

Received: 26 July 2021
Accepted: 21 October 2021
Published: 09 November 2021

Citation:
Melo-González F, Soto JA, González LA, Fernández J, Duarte LF, Schultz BM, Gálvez NMS, Pacheco GA, Ríos M, Vázquez Y, Rivera-Pérez D, Moreno-Tapia D, Iturriaga C, Vallejos OP, Berríos-Rojas RV, Hoppe-Elsholz G, Urzúa M, Bruneau N, Fasce RA, Mora J, Grifoni A, Sette A, Weiskopf D, Zeng G, Meng W, González-Aramundiz JV, González PA, Abarca K, Ramírez E, Kalergis AM and Bueno SM (2021) Recognition of Variants of Concern by Antibodies and T Cells Induced by a SARS-CoV-2 Inactivated Vaccine. Front. Immunol. 12:747830. doi: 10.3389/fimmu.2021.747830

against Spike from variants of concern were evaluated in PBMCs from vaccinated subjects using ELISPOT.

Results: CoronaVac promotes the secretion of antibodies able to block the RBD of all the SARS-CoV-2 variants studied. Seropositivity rates of neutralizing antibodies in the population evaluated were over 97% for the lineage B strain, over 80% for Alpha and Gamma variants, over 75% for Delta variant and over 60% for the Beta variant. Geometric means titers of blocking antibodies were reduced when tested against SARS-CoV-2 variants as compared to ancestral strain. We also observed that antibodies from vaccinated subjects were able to neutralize the infection of variants D614G, Alpha, Gamma and Delta in a conventional microneutralization assay. Importantly, after SARS-CoV-2 infection, we observed that the blocking capacity of antibodies from vaccinated volunteers increased up to ten times for all the variants tested. We compared the number of interferon-γ-secreting T cells specific for SARS-CoV-2 Spike WT and variants of concern from vaccinated subjects and we did not detect significant differences.

Conclusion: Immunization with CoronaVac in either immunization schedule promotes the secretion of antibodies able to block SARS-CoV-2 variants of concern and partially neutralizes SARS-CoV-2 infection. In addition, it stimulates cellular responses against all variants of concern.

Keywords: CoronaVac, SARS-CoV-2, antibodies, vaccine, variants of concern, T cell immunity

INTRODUCTION

SARS-CoV-2 represents a global threat to public health and has been responsible for over 4 million deaths worldwide to date (1). After the spread of the original wild-type SARS-CoV-2 strain, multiple mutants have arisen around the world. Most of these circulating variants belong to the SARS-CoV-2 lineage B, in particular lineage B.1 (2). One of the most prevalent strains is the D614G, which displays a mutation in the C-terminal region of the Spike 1 (S1) domain outside the Receptor Binding Domain (RBD) (2). Although this mutant has been reported to be more infective, sera from convalescent patients and subjects vaccinated with mRNA vaccines are able to neutralize the D614G mutant to an extent similar to that of the ancestral strain, i.e. lineage B or wild type strain (2–5).

Current vaccination programs around the world are facing the threat of these circulating variants of concern of SARS-CoV-2, as they exhibit different mutations in the RBD and may evade antibody neutralization (2). To facilitate their identification, variants of concern are currently termed Alpha (B.1.1.7), Beta (B1.351), Gamma (P.1), and Delta (B.617.2) (6). Alpha (first identified in the UK), Beta (first identified in South Africa) and Gamma (first identified in Brazil) mutants share the N501Y mutation that has been linked with increased affinity of the Spike protein for the endogenous receptor human Angiotensin-converting enzyme 2 (hACE2) (7). Beta and Gamma mutants exhibit the E484K mutation, associated with an increased evasion of neutralizing antibodies (8–10). Furthermore, Beta and Gamma exhibit mutations in the residue K417 of the RBD but differ in the amino acid substitutions (K417N for Beta and K417T for Gamma), which may affect antibody binding (6). In addition, the Delta variant (first identified in India) is currently a cause of concern due to its high transmissibility and may even surpass other variants in this regard (11). Delta exhibits unique mutations (L452R, T478K and P681R), which may increase viral infectivity and viral fusion (12, 13). Considering the increased infectivity and death rates described for these variants, it is crucial to understand whether vaccination can induce protection against them (6).

Chile is among the countries with the highest percentage of vaccination worldwide (over 56% of the total population), and CoronaVac, an inactivated SARS-CoV-2 vaccine, represents 78.2% of the immunized population (14). A phase 3 clinical trial is being conducted in Chile, with two vaccination schedules: two doses separated by 14 days (0-14) or by 28 days (0-28), and the general population has received the latter schedule. CoronaVac is safe and induces humoral and cellular responses in vaccinated subjects from different age groups, and has been proven effective in remarkably reducing hospitalizations and death rates (15, 16). Here, we evaluate the blocking and neutralizing capacities of circulating antibody induced by CoronaVac in vaccinated volunteers for both schedules against the most prevalent variants in Chile. Blocking capacities against the RBD of variants Alpha, Beta, Gamma and Delta were tested with an in-house surrogate neutralization test (sVNT) and compared to the wild strain, included in the vaccine formulation. The neutralizing capacities of antibody were evaluated using a conventional plaque-reduction neutralization test (cVNT) for the D614G, Alpha, Gamma and Delta variants. Our data shows that vaccinated volunteers exhibit circulating

antibodies with neutralizing capacities against the different variants of concern, with a better response against the Alpha and Gamma variants, although inhibition of the binding between hACE2 and RBD from the Beta variant was also detected using sVNT. We also observed that CoronaVac promotes Interferon-γ (IFN-γ)-producing $CD4^+$ T cells against Spike peptides from variants of concern. These results suggest that the antibodies and cellular responses induced by the administration of two doses of CoronaVac would have a protective role against the several circulating variants of concern of SARS-CoV-2.

METHODS

Study Design and Volunteers

The clinical trial (clinicaltrials.gov NCT04651790) was conducted in Chile at eight different sites and evaluated two immunization schedules. This trial was approved by each Institutional Ethical Committee and the Chilean Public Health Institute (#24204/20) and conducted according to the current Tripartite Guidelines for Good Clinical Practices, the Declaration of Helsinki (17), and local regulations. Volunteers were inoculated with either two doses of 3 μg (600SU) of CoronaVac at 0- and 14-days or 0- and 28-days post the first immunization (p.i.). Written informed consent was obtained from each participant. Exclusion criteria included history of confirmed symptomatic SARS-CoV-2 infection, pregnancy, allergy to vaccine components, and immunocompromised conditions. A complete list of inclusion and exclusion criteria has been published previously (15). A total of 2,302 volunteers were enrolled by March 19th, 2021, and a subgroup of 440 volunteers was chosen to evaluate their immune response. Demographic information, co-morbidities, nutritional status, immunization schedule, and dates of vaccination, were obtained at enrolment for all volunteers.

Procedures

Sera samples from the 0-14 and 0-28 immunization schedules were chosen among those that were previously confirmed as positive against wild-type SARS-CoV-2 through commercial kits (GenScript #L00847-A and BioHermes #COV-S41). A total of 42 samples (22 samples from the 0-14 schedule and 20 from the 0-28 schedule) were evaluated by sVNT. A total of 52 samples (34 samples from the 0-14 schedule and 18 samples from the 0-28 schedule) were evaluated by cVNT. Both groups included volunteers aged 18 to 59 years and over 60 years.

To assess the capacity of the antibodies against SARS-CoV-2 circulating variants of concern to inhibit RBD and hACE2 interaction in the samples from vaccinated volunteers, we performed in-house SARS-CoV-2 sVNT based on previous reports (18). RBD unconjugated proteins from wild-type (WT) SARS-CoV-2 (GenScript #Z03483) and the variants B.1.1.7 (GenScript #Z03533), B.1.351 (GenScript #Z03537) P.1 (SinoBiological #40592-V08H86) and B.1.617.2 (GenScript #Z03613) were conjugated to HRP using the HRP Conjugation Kit - Lightning Link (#ab102890) in a 2:1 mass ratio (HRP to RBD) following the instructions of the manufacturer. ELISA 96-well plates (SPL) were pre-coated with 100 ng per well of the recombinant hACE2 protein (GenScript #Z03484) in 50 μL of 100 mM carbonate–bicarbonate coating buffer (pH 9.6) ON at 4°C. Plates were then washed three times with PBS - 0.05% Tween 20 and blocked with PBS - 10% FBS for 2h at RT. The HRP-RBD conjugates obtained previously were then incubated with the serum sample in a final volume of 120 μL for 1 h at 37°C. Concentration of conjugates used were as follows: 3 ng of WT SARS-CoV-2, 0.75 ng of B.1.1.7, 3 ng of B.1.351, 3 ng of P.1 and 3 ng of B.1.617.2. Then, these mixtures were added into the 96-well plates coated with hACE2 and were incubated for 1 h at RT. Unbound HRP-RBD were removed washing five times with PBS - 0.05% Tween 20. Then, 50 μL of 3,3',5,5'-tetramethylbenzidine (TMB – BD #555214) was added. An equal volume of 2 N H_2SO_4 was added to stop the reaction, and optical densities (OD) values at 450 nm were read. The antibody titer was determined as the last fold-dilution with a cut-off value over 20% of inhibition. The percentage of inhibition was defined as: [OD_{450nm} value of negative control-OD_{450nm} value of sample]/[OD_{450nm} value of negative control*100]. Negative controls (corresponding to sera sample obtained before immunization) were included. For the cVNT, sera samples were two-fold serially diluted starting at a 4-fold dilution until a 512-fold. Then, samples were incubated for 1 h at 37°C with an equal volume of a SARS-CoV-2 33782CL-SARS-CoV-2 strain (lineage B, D614G), Alpha (B.1.1.7), Gamma (P.1) and Delta (B.1.617.2) variants. These variants were previously isolated by the Institute of Public Health of Chile from clinical samples. These mixtures were inoculated on confluent Vero E6 cell monolayers (ATCC CRL-1586) and cytopathic effect (CPE) was evaluated seven days later. Sera samples from uninfected patients (negative controls) and sera samples from confirmed COVID-19 patients (positive controls) were included. Plaque forming units were quantified by direct visualization and the titer of neutralizing antibodies was defined as the highest serum dilution that neutralized 100% of virus infection. Seropositivity rates were calculated as the percentage of the population evaluated that showed end titers ≥1/4 in both techniques.

To assess the cellular immune response, ELISPOT assays were performed using PBMCs from 18 participants, as described previously, using the human IFN-γ/interleukin-4 (IL-4)double-color ELISPOT (Immunospot) (15). Cells were stimulated for 48h in the presence of Mega Pools (MPs) of peptides derived from SARS-CoV-2 Spike WT, Alpha, Beta, Gamma and Delta at 37°C, 5% CO_2. As positive controls, an independent stimulation performed with 5 mg/mL of Concanavalin A (ConA) (Sigma Life Science #C5275-5MG) and with an MP of peptides derived from cytomegalovirus proteins (MP-CMV) for the stimulation of both $CD4^+$ and $CD8^+$ T cells. As a vehicle control, DMSO 1% (Merck #317275) was included. Spot Forming Cells (SFCs) were counted on an ImmunoSpot® S6 Micro Analyzer.

Statistical Analysis

Statistical differences were evaluated by Wilcoxon tests (for comparisons between two groups). Differences were considered

significant if the p value was under 0.05. All data were analyzed with GraphPad Prism 9.0.1.

RESULTS

To assess whether volunteers from the Phase 3 clinical trial being held in Chile exhibited antibodies able to inhibit the RBD of SARS-CoV-2 circulating variants of concern, we performed an in-house sVNT designed to evaluate the inhibition of the interaction between hACE2 and RBD, which has been previously shown to correlate with neutralizing antibodies (15, 18). Samples from volunteers immunized with two doses of CoronaVac in a 0-14 or 0-28 immunization schedule were tested. Levels of antibodies able to inhibit the interaction between hACE2 and RBD from circulating SARS-CoV-2 variants of concern combining both 0-14 and 0-28 immunization schedules are shown in **Figure 1A**. We report a 1.8-fold reduction of antibody titers that inhibit the variant Alpha, a 5.9-fold reduction of titers against the variant Beta, a 3-fold reduction of titers against the variant Gamma, and a 3.5-fold reduction of titers against the variant Delta, as compared to the WT strain. These reductions were associated with a decrease in GMT values, i.e., 29.5 (95% CI 20.1-43) for the WT strain, 16.0 (95% CI 10.9-23.5) for Alpha, 5.0 (95% CI 3.8-6.7) for Beta, 9.8 (95% CI 6.9-13.9) for Gamma, and 8.5 (95% CI 6.1-11.9) for Delta. Reductions seen for variants Beta, Gamma, and Delta were detected in both age groups. Interestingly, participants aged 18-59 years did not exhibit significant differences in the level of antibodies inhibiting the WT strain and the Alpha variant (**Supplementary Figure 1**). The seropositivity rate of the neutralizing antibodies in the population evaluated was 100% for the WT strain and 88.1%, 64.2%, 88.1% and 78.6% for Alpha, Beta, Gamma, and Delta, respectively.

For the 0-14 immunization schedule, antibodies that inhibit the variants Alpha, Beta, and Gamma were measured 28 days after administration of the second dose. GMTs of antibodies able to inhibit the RBDs (**Figure 1B**) are lower compared to the wild-type strain (17.6, 95% CI 10.2-30.1) and the lowest reported value were against the Beta variant (GMT 4.8, 95% CI 3.1-7.4, a 3.6-fold reduction) and Delta variant (GMT 7.8, 95% CI 4.7-12.9, a 2.3-fold reduction). In contrast, similar GMT values were found for the Alpha and Gamma variants (12.8, 95% CI 7.7-21.5 and 12.4, 95% CI 7.3-21.2, respectively). Similar values were found when samples were analyzed according to their age group, although volunteers aged 18 to 59 years old exhibited a significant decrease in antibodies against the Beta RBD and Delta RBD whereas volunteers over 60 years only exhibit a significant decrease against the Beta RBD (**Supplementary Figures 2A, B**). The seropositivity rate was 95.45% of the evaluated volunteers exhibiting neutralizing antibodies against the WT strain, while the percentages against the Alpha, Beta, Gamma and Delta variants were 86.36%, 63.64%, 86.36%, and 72.72%, respectively.

For volunteers of the 0-28 immunization schedule, increased GMT values in antibodies able to block the RBDs were found against the WT strain (52.0, 95% CI 33.2-81-3) compared to the GMTs for the WT strain observed in the 0-14 schedule, as observed in Fig 1C. These GMT values decreased when evaluating the circulating variants of concern (Alpha, 2.5-fold reduction, GMT 20.4, 95% CI 11.1-37.4; Beta, 9.8-fold reduction, GMT 5.3 95% CI 3.4-8; Gamma, 6.9-fold reduction, GMT 7.5, 95% CI 4.7-11.9; and Delta, 5.5-fold reduction, GMT 9.5 95% CI 5.9-15.4) (**Figure 1C**). Decreases in GMT values against the Beta, Gamma and Delta variants were seen for both age groups in this immunization schedule. However, volunteers aged 18-59 years exhibited a similar GMT between the WT strain and the Alpha variant (**Supplementary Figures 2C, D**). Seropositivity rates of antibodies measured for this schedule are showed in **Figure 1C** and are similar to those reported for the 0-14 schedule. The results indicate that 100% of the evaluated volunteers exhibited antibodies able to inhibit the WT strain, while percentages against the Alpha, Beta, Gamma, and Delta variants were 90%, 65%, 80% and 85%, respectively.

In order to further corroborate whether these antibodies were also able to neutralize viral infection in a cell culture, we performed cVNT for lineage B SARS-CoV2 (D614G) and the Alpha, Gamma, and Delta variants. The results obtained showed that, as compared to the D614G strain, there was a 2.33-fold decrease in neutralizing antibodies against the Alpha variant, a 4.73-fold reduction against the Gamma variant and a 9.46-fold reduction against the Delta variant (**Figure 2A**). This result suggests that CoronaVac induce the secretion of antibodies that can neutralize these variants, but at rates lower than those reported for the WT or the D614G strain. The GMT values obtained by cVNT for D614G strain and the Alpha, Gamma, and Delta variants were 74.8 (95% CI 59.8-93.6), 32.1(95% CI 20.1-51.1), 15.8 (95% CI 9.5-26.2) and 7.9 (95% CI 5.2-12), respectively. As also seen for sVNT, volunteers aged 18 to 59 years exhibit a significant decrease in neutralizing antibodies against Gamma, and Delta, whereas volunteers over 60 years old exhibited significantly decreased neutralizing antibodies against Alpha and Delta and a lower but insignificant decrease in neutralizing antibodies against Gamma (**Supplementary Figure 3**). The seropositivity rates of neutralizing antibodies for the Alpha, Gamma and Delta variants were 84.62%, 65.38% and 55.76% respectively, while for the D614G strain was 97.6% (**Figure 2B**). Further details regarding the values reported on **Figures 1** and **2** can be found in **Tables 1** and **2**.

We also evaluated whether nine volunteers infected with SARS-CoV-2 after their respective vaccination schedules were completed (breakthrough cases) produced antibodies inhibiting the RBDs of the different variants evaluated. **Figure 3** compares antibodies levels 28 days after the second dose of CoronaVac (pre-infection) and 28 days after the infection were detected (post-infection). Most of the volunteers exhibited a 10-fold increase in the GMT of antibodies able to inhibit the RBDs of the four variants evaluated (Alpha, Beta, Gamma and Delta), as compared to GMT observed for samples previous infection. Therefore, natural infection with SARS-CoV-2 increases the secretion of antibodies that can block the interaction of RBDs from the Beta, Gamma, and Delta variants with the hACE2

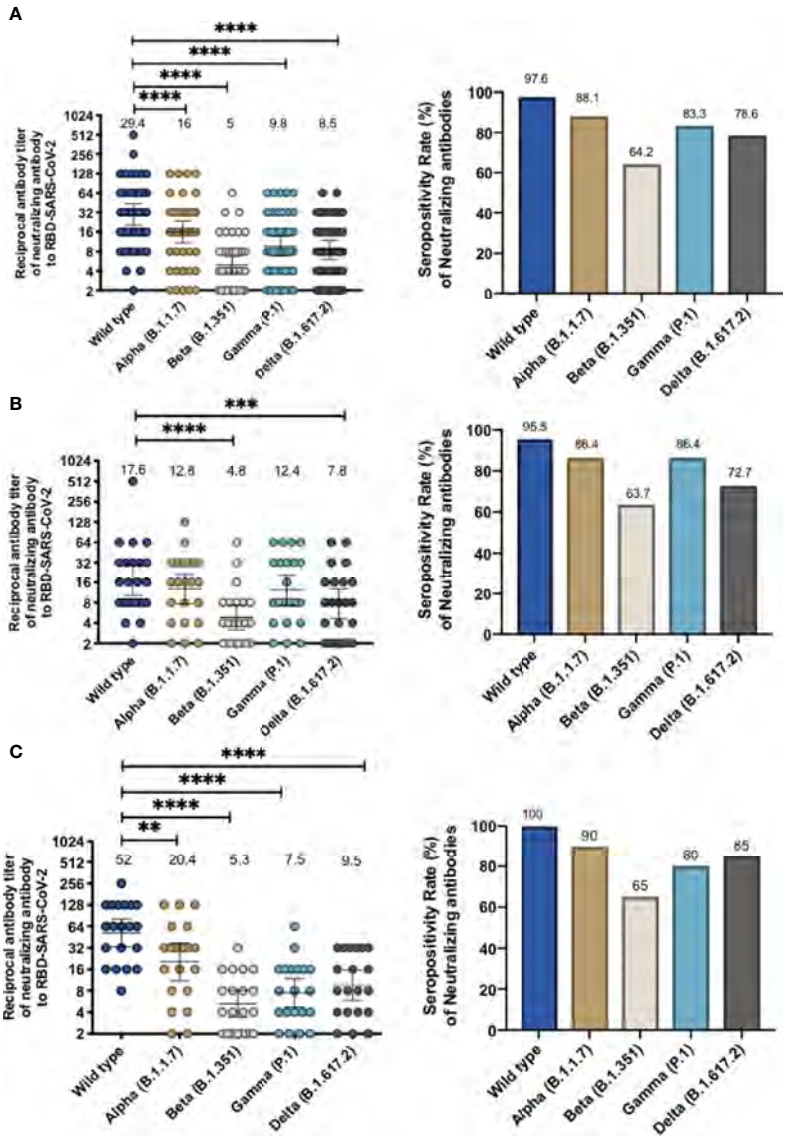


FIGURE 1 | Immunization with CoronaVac induces antibodies able to inhibit the interaction between hACE2 and S1-RBD from SARS-CoV-2 variants after two immunizations in a 0-14 and 0-28 schedule. Antibody titers were evaluated with a surrogate virus neutralization assay (sVNT), which quantifies the interaction between S1-RBD from either WT SARS-CoV-2 or variants of concern (Alpha, Beta, Gamma, and Delta) and hACE2 on ELISA plates. Total neutralizing antibodies titer from volunteers vaccinated with CoronaVac, 28 days after the second dose and the seropositivity rate of neutralizing antibodies are shown for both vaccination schedules **(A)**, 0-14 schedule **(B)** and 0-28 schedule **(C)**. Numbers above the bars show the Geometric Mean Titer (GMT), and the error bars indicate the 95% CI in the graphs showing total antibody titers, and the number above bars show the percentage of seropositivity rate in the respective graphs. A Wilcoxon test analyzed data to compare against the wild-type RBD; $^{**}p < 0.005$, $^{***}p < 0.001$, $^{****}p < 0.0001$. The graph represents the results obtained for 22 volunteers for the 0-14 schedule and 20 volunteers for the 0-28 schedule.

receptor. However, further analyses are still required, as no characterization of the variants infecting these volunteers was performed.

Moreover, we have recently shown that CoronaVac is able to stimulate $CD4^+$ T cell responses against MPs of both Spike and Non-Spike peptides, displaying higher secretion of IFN-γ and expression of activation markers following vaccination in a 0-14 schedule, which peaks 14 days after the second dose (15). In order to evaluate anti-Spike $CD4^+$ T cell responses, we stimulated PBMCs of participants from both 0-14 and 0-28 schedules with Spike MPs from the WT strain and variants of concern and evaluated IFN-γ expression by ELISPOT (**Figure 4**). As previously reported, the subjects evaluated exhibited robust IFN-γ production following stimulation and we did not observe significant differences between PBMCs stimulated with any of the Spike MPs, suggesting that CoronaVac induces protective

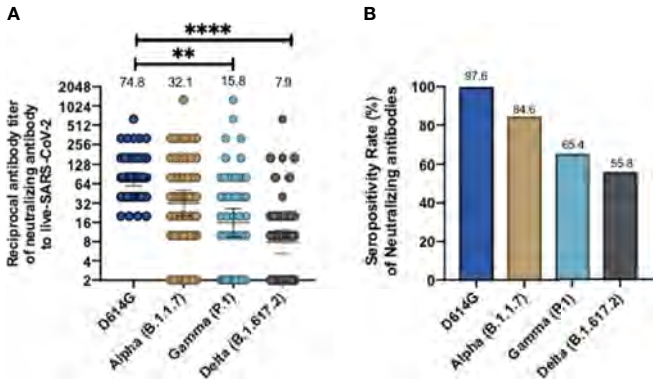


FIGURE 2 | CoronaVac immunization induces neutralizing antibodies against SARS-CoV-2 variants after two vaccine doses using a conventional virus neutralization test. Neutralizing antibody titers were evaluated by incubating the serum with a SARS-CoV-2 Chilean clinical strains and then added into Vero E6 cell for seven days. The neutralizing titer was determinate for the last dilution where no viral cytopathic effect was found in cells against wild type (D614G), and Alpha, Gamma and Delta variants. Consolidate neutralizing antibodies titer of both schedules is shown in **(A)**, and the seropositivity rate of neutralizing antibodies is shown in **(B)**. Numbers above the bars show the Geometric Mean Titer (GMT), and the error bars indicate the 95% CI in **(A)**, and the number above bars in **(B)** showed the seropositivity rate. A Wilcoxon test analyzed data to compare against the wild-type RBD; **p < 0.005, ****p < 0.0001. The graph represents the results obtained for 52 volunteers of both schedules.

cellular responses against all SARS-CoV-2 variants of concern. In addition, we observed low numbers of IL-4-secreting T cells in response to all of the MPs (**Supplementary Figure 4**), which is consistent with our previous data using the MP-S WT.

DISCUSSION

The current spread of multiple SARS-CoV-2 variants worldwide challenges the strategies of vaccination and represent a threat for potential new waves of infection. The inactivated SARS-CoV-2 vaccine CoronaVac has been proven to induce total IgG and neutralizing antibodies against the Spike protein in subjects vaccinated with either a 0-14 or 0-28 vaccination schedule, although those levels are lower as compared to other vaccines such as BNT16b2 and Moderna mRNA-1273 (15, 19, 20). Here we report that CoronaVac induces the secretion of neutralizing antibodies that recognize most of the variants of concern currently circulating in the population, as determined by sVNT and cVNT (**Figures 1**–**3**). Although the intrinsic characteristics for each of the techniques used in this report to evaluate circulating neutralizing antibodies in immunized volunteers were different, the results obtained were mostly equivalent for the WT strain, as described in our previous studies (15, 21). We found similar fold reductions in blocking and neutralizing antibodies against the variants Alpha and Gamma using both techniques, but a higher fold reduction against the Delta variant (3.5-fold reduction in the sVNT and 9.46-fold reduction in the cVNT) was observed. Moreover, when evaluating through cVNT, lower seropositivity rates were observed against the Gamma and Delta variants (65.4% and 55.8%, respectively) as compared to the results obtained by sVNT (83.3% and 78.57%, respectively), but we report a similar percentage of seropositivity for participants with circulating neutralizing antibodies against at least two of the variants with both techniques (88.1% by sVNT and 78.8 by cVNT) (**Tables 1** and **2**). These results are in line with previous reports that have shown a high correlation between these two techniques (15, 18). A recent study that

TABLE 1 | Seropositivity rates and geometric mean titer of antibodies that inhibit the RBDs of SARS-CoV2 variants, by sVNT.

Schedule	Indicators	Wild type	Alpha (B.1.1.7)	Beta (B.1.351)	Gamma (P.1)	Delta (B.1.617.2)	Seropositivity rate over 2 variants
0-14	Seropositivity n/N	21/22	19/22	14/22	19/22	16/22	19/22
	(%)	95.5	86.4	63.6	86.4	72.72	86.4
	GMT	17.6	12.8	12.4	4.8	7.8	N/D
	(95% CI)	10.3-30.2	7.7-21.5	7.3-21.2	3.2-7.4	4.7-12-9	(-)
0-28	Seropositivity n/N	20/20	18/20	13/20	16/20	17/20	18/20
	(%)	100	90.0	65.0	80.0	85.0	90.0
	GMT	52.0	20.4	7.5	5.3	9.5	N/D
	(95% CI)	33.1-81.4	11.1-37.4	4.7-11.2	3.4-8.1	5.9-15.4	(-)
Total	Seropositivity n/N	41/42	37/42	27/42	35/42	33/42	37/42
	(%)	97.6	88.1	64.3	83.3	78.57	88.1
	GMT	29.5	16.0	9.8	5.0	8.5	N/D
	(95% CI)	20.2-43.1	10.9-23.5	6.9-13.9	3.8-6.7	6.1-11.9	(-)

RBD, Receptor-binding domain; S, Spike; GMT, Geometric mean titer; N/D, Not determined.

TABLE 2 | Seropositivity rates and geometric mean titer of neutralizing antibodies against SARS-CoV2 variants by cVNT.

Schedule	Indicators	D614G	Alpha (B.1.1.7)	Gamma (P.1)	Delta (B.1.617.2)	Seropositivity rate over 2 variants
0-14	Seropositivity n/N	34/34	27/34	27/34	20/34	29/34
	(%)	100	79.4	79.4	58.8	85.2
	GMT	57.7	26.5	27.0	7.7	N/D
	(95% CI)	45.1-74.0	14.9-47.1	14.8-49.4	4.7-12-6	(-)
0-28	Seropositivity n/N	18/18	17/18	7/18	9/18	12/18
	(%)	100	94.4	38.9	50.0	66.6
	GMT	122.2	46.1	5.7	8.3	N/D
	(95% CI)	83.9-178.1	19.8-107.2	2.6-12.4	3.5-19.7	(-)
Total	Seropositivity n/N	52/52	44/52	34/52	29/52	41/52
	(%)	100	84.6	65.4	55.8	78.8
	GMT	74.8	32.1	15.8	7.9	N/D
	(95% CI)	59.8-93.6	20.1-51.1	9.5-26.2	5.2-12	(-)

GMT, Geometric mean titer; N/D, Not determined.

used the sVNT and cVNT to evaluate neutralizing antibodies against SARS-CoV-2 variants of concern in heterologous and homologous ChAdOx1 nCoV-19/BNT162b2 vaccination has shown high correlation between both assays (22).

Our results are in line with the effectiveness of CoronaVac observed in a study of elderly subjects vaccinated in Brazil, where the Gamma variant is the most prevalent SARS-CoV-2 strain and an effectiveness of 42% was reported (23). Furthermore, our data is consistent with a recent study in volunteers vaccinated with two doses of CoronaVac in China, which exhibit a 4.3-fold reduction of VNT in live neutralization assays against the Gamma variant compared to the WT strain and another study with individuals vaccinated with two doses of CoronaVac in Brazil, which reported reduced VNT against the isolates P.1/28 and P.1/30 as compared to the WT strain (a 3.1 and 2.6 fold reduction, respectively) (24, 25). Similarly, here we report a 4.73 fold reduction compared to the D614G strain using cVNT (**Figure 2**). In addition, other studies carried out in Chile using cVNT and pseudotyped viruses have reported a 7.51 and 2.33-fold reduction, respectively, in Gamma variant neutralization as compared to the WT strain in subjects vaccinated with CoronaVac (26, 27). The reduced neutralizing capacities reported against the Gamma variant have been related to the E484K mutation, which promotes the evasion of neutralizing antibodies (28). Importantly, the Gamma variant became one of the dominant SARS-CoV-2 strains in Chile during 2021 in parallel to the vaccination of Chilean population with CoronaVac (26). However, only 45 out of 2,263 participants of the phase 3 clinical trial carried out in Chile developed breakthrough cases following vaccination and among these individuals 96% developed mild disease, which suggests that CoronaVac is protective against SARS-CoV-2 and potentially against SARS-CoV-2 variants (21).

We also reported neutralizing responses against the Beta variant in subjects vaccinated with two doses of CoronaVac. A reduced inhibition of the interaction between hACE2 and RBD compared to the WT strain and a seropositivity of 64.2% was

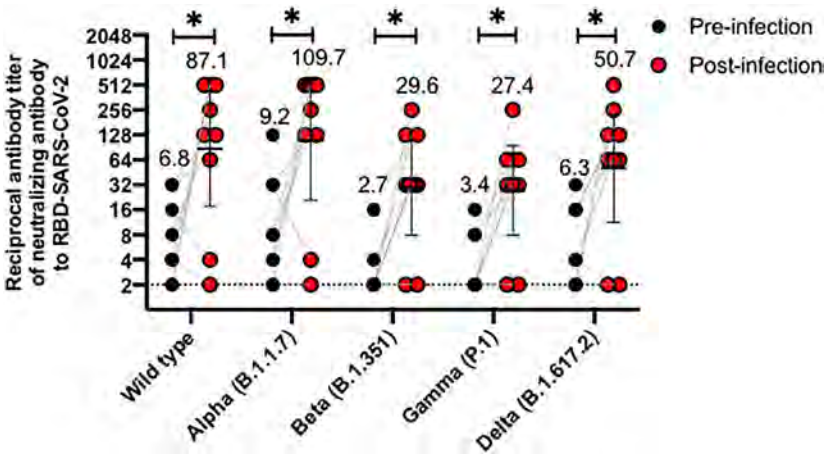


FIGURE 3 | CoronaVac immunization induces antibodies able to inhibit the interaction between hACE2 and S1-RBD from SARS-CoV-2 variants in vaccine breakthrough cases after two vaccine doses. Antibody titers were evaluated with a surrogate virus neutralization assay (sVNT), which quantifies the interaction between S1-RBD from either Wild type SARS-CoV-2 or variants of concern (Alpha, Beta, Gamma, and Delta) and hACE2 on ELISA plates. Comparative data from vaccine breakthrough cases from both schedules are represented for each variant in two different point times, pre-infection (black circle) and post-infection (red circles). A Wilcoxon test analyzed data to compare against the wild-type RBD; *$p < 0.05$. The graph represents the results obtained for nine volunteers considering both schedules.

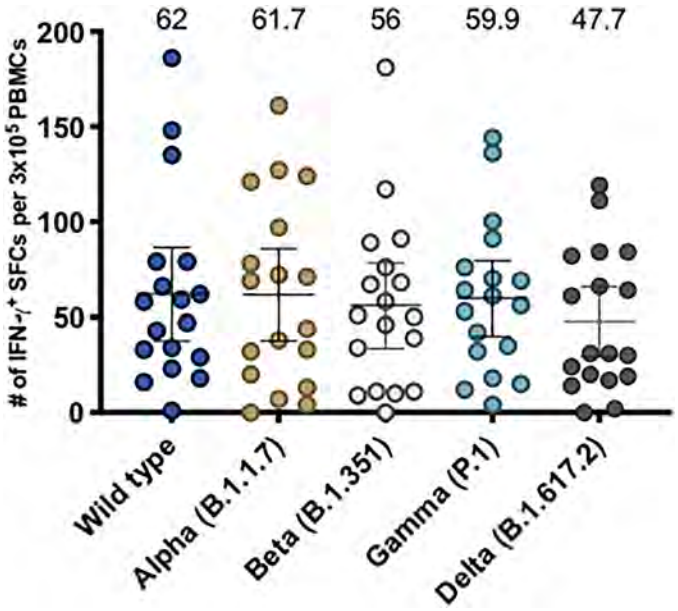


FIGURE 4 | Evaluation of cellular immune response through ELISPOT upon stimulation with Mega Pools of Spike peptides derived from SARS-CoV-2 WT and variants of concern in volunteers immunized with CoronaVac. Numbers of IFN-γ-secreting cells, determined through ELISPOT as spot forming cells (SFCs) were determined. PBMCs were stimulated with MP-S WT, MP-S Alpha, MP-S Beta, MP-S Gamma and MP-S Delta for 48 h for samples obtained 2 weeks after the second dose of volunteers of the 0-14 schedule (n = 11) and 0-28 schedule (n = 7). A total of 18 volunteers were evaluated. Data shown represents mean ± 95% CI and the mean is indicated above each bar. Statistical differences were evaluated by a one-way ANOVA followed by Dunnett's test for multiple comparisons against the MP-S WT.

reported using the sVNT, the lowest across all variants of concern analyzed (**Figure 1** and **Table 1**). These results are consistent with recent reports in cohorts from Thailand and China vaccinated with CoronaVac, in which reduced neutralization was reported using live virus neutralization (fold reductions of 22.1 and 5.7 compared to the WT strain, respectively) (24, 29) and also with the reduction in neutralizing responses observed in subjects vaccinated with the mRNA vaccine BNT162b2 for the Beta variant (4, 30). In line with the reports for the Gamma variant, the E484K mutation found in the Beta variant has been identified as the main mutation responsible for this effect as antibodies bind to RBD with less affinity.

Of note, we used the D614G variant in the cVNT, which exhibits a mutation outside of the RBD and we were able to observe effective neutralization against viral infection in all the subjects evaluated from both vaccination schedules and both age groups (**Figure 2**). These results support that CoronaVac is protective against the D614G variant, which is one of the most prevalent strains worldwide.

Our work also reported protection against the variant Delta. The Delta variant (first identified in India) exhibit the RBD mutations T478K, L452R and P681R and is currently a cause of concern due to its high transmissibility and may even surpass other variants in this regard (11). The Delta variant has been recently detected in Chile and it is becoming one of the dominant SARS-CoV-2 strains. Here we show using a RBD containing the mutations T478K and L452R present in the Delta variant that volunteers vaccinated with CoronaVac exhibit reduced blocking antibodies compared to the WT RBD but we report a seropositivity of 78.57% and 55.76% by sVNT and cVNT (**Tables 1** and **2**), respectively, which suggests that the vaccine confers protection against this variant. Our data is in line with the previously mentioned works from Thailand and China in volunteers vaccinated with 2 doses of CoronaVac, in which neutralization was evaluated by cVNT and reported fold reductions of 31.7 and 3.7 fold reduction, respectively, as compared to the WT strain, whereas we report a 9.46-fold reduction (24, 29). Similarly, mRNA vaccines induce neutralizing antibodies against the Delta variant but to a reduced extent compared to the WT strain (31, 32). Pseudoviruses carrying the L452R mutation display higher infectivity in cell culture and when incubated with sera from subjects vaccinated with Moderna mRNA-1273 or BNT16b2, as compared to the WT strain (13).

Our study also shows how subjects vaccinated with CoronaVac increase their blocking antibody GMTs following natural infection against the wild type strain and to a similar extent to the Alpha variant, but this increased GMT was lower for the variants Beta, Gamma and Delta (**Figure 3**). These findings are consistent with studies comparing different vaccine platforms against natural infection, which indicate that inactivated vaccines induce lower levels of neutralizing antibodies compared to natural infection with SARS-CoV-2, in contrast to mRNA vaccines, which exhibit comparable levels of neutralization, using live virus neutralization (20). In line with

this, cohorts from Thailand and Brazil vaccinated with CoronaVac exhibits lower neutralizing antibody titers against either the WT strain or variants of concern, compared to naturally infected individuals (25, 29). We have previously reported levels of neutralization in unvaccinated and naturally infected hospitalized individuals, which exhibit a robust neutralizing antibody response against wild-type SARS-CoV-2 (33). Although we did not perform cVNT for either breakthrough cases or naturally infected individuals against variants of concern, our results obtained by sVNT are in line with data from non-variant infected subjects, who also exhibit a similar reduction in neutralization against the variants Beta, Gamma and Delta (20).

Moreover, here we show that CoronaVac is able to stimulate T cell responses against Spike MPs from either WT strain or variants of concern and we did not see any significant differences (**Figure 4**). This is the first report to date to characterize T cell responses against SARS-CoV-2 Spike MPs in volunteers vaccinated with CoronaVac. Concordantly, MPs from variants of concern have been previously used to show that volunteers vaccinated with two doses of either Moderna mRNA-1273 or BNT16b2 exhibit IFN-γ-secreting T cells in response to these MPs and no significant differences were found (34). These results have been attributed to the high conservation of T cell epitopes in variants of concern, suggesting that vaccines can induce effective cellular responses against them. In addition, it is important to highlight that although the majority of the T cell responses are conserved and the variants do not mutate enough to disrupt the overall T cell repertoire, mutations are observed in other SARS-CoV-2 proteins and across variants (34). Therefore, it is likely that the induction of cellular responses against other SARS-CoV-2 proteins by CoronaVac may confer an advantage compared to other vaccines, considering that the inclusion of multiple antigens might increase the likelihood that more epitopes are conserved than having only one protein in the vaccine.

Importantly, a limitation of our study is that we were not able to characterize other non-neutralizing antibody functions that could be important in either vaccinated or convalescent subjects against variants of concern. Furthermore, *in vitro* evaluation of neutralizing antibodies does not necessarily correlate with protection against SARS-CoV-2 in vaccinated individuals. However, recent evidence supports that levels of neutralizing antibodies are predictive of protection against symptomatic SARS-CoV-2 infection (35). In addition, although cellular responses do not necessarily prevent infection, induction of cellular responses against variants of concern in individuals vaccinated with CoronaVac suggests that vaccinated individuals are protected from severe disease, which is supported from the results of the clinical trial performed in Chile with this vaccine (16, 21).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This trial was approved by each Institutional Ethical Committee and the Chilean Public Health Institute (#24204/20) and conducted according to the current Tripartite Guidelines for Good Clinical Practices, the Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization and visualization, AK, ER, SB, KA, PG, and JG-A. Methodology, RF, JM, JF, GZ, WM, AG, AS, and DW. Investigation, FM-G, JS, JF, NB, LG, BS, LD, NG, GAP, RB-R, GH-E, CI, DM-T, MR, DR-P, OV, MU, and YV. Funding acquisition, AK. Project administration, AK, KA, SB, PG, and JG-A. Supervision, AK, KA, SB, and PG. Writing – original draft, FM-G and JS. Writing – review and editing, AK, KA, SB, ER, PG, AG, AS, and DW. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by The Ministry of Health, Government of Chile supported the funding of the CoronaVac03CL Study, The Confederation of Production and Commerce (CPC), Chile, supported the funding of the CoronaVac03CL Study, the NIH NIAID under Contract No. 75N93021C00016 supports AS and Contract No. 75N9301900065 supports AS, DW. The Millennium Institute on Immunology and Immunotherapy, ANID - Millennium Science Initiative Program ICN09_016 (former P09/016-F) supports SB, KA, PG, and AK. The Innovation Fund for Competitiveness FIC-R 2017 (BIP Code: 30488811-0) supports SB, PG, and AK. SINOVAC contributed to this study with the investigational vaccine and experimental reagents.

ACKNOWLEDGMENTS

We would like to thank the support of the Ministry of Health, Government of Chile, Ministry of Science, Technology, Knowledge, and Innovation, Government of Chile, and The Ministry of Foreign Affairs, Government of Chile and the Chilean Public Health Institute (ISP). We also would like to thank Rami Scharf, Jessica White, Jorge Flores and Miren Iturriza-Gomara from PATH for their support on experimental design and discussion. We also thank the Vice Presidency of Research (VRI), the Direction of Technology Transfer and Development (DTD), the Legal Affairs Department (DAJ) of the Pontificia Universidad Católica de Chile. We are also grateful to the Administrative Directions of the School of Biological Sciences and the School of Medicine of the Pontificia Universidad Católica de Chile for their

administrative support. Special thanks to the members of the CoronaVac03CL Study Group [list in the Supplementary Appendix (SA)] for their hard work and dedication to the clinical study; the members of the independent data safety monitoring committee (members in the SA) for their oversight, and the subjects enrolled in the study for their participation and commitment to this trial.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.747830/full#supplementary-material

Supplementary Figure S1 | Immunization with CoronaVac induces antibodies able to inhibit the interaction between hACE2 and S1-RBD from SARS-CoV-2 variants in participants aged 18-59 and ≥60 after two immunizations. Antibody titers were evaluated with a surrogate virus neutralization assay, which quantifies the interaction between S1-RBD from either Wild type SARS-CoV-2 or variants of concern (Alpha, Beta, Gamma and Delta) and hACE2 on ELISA plates. Results were obtained from participants vaccinated with CoronaVac, 28 days after the second dose in volunteers between 18-59 **(A)** and ≥ 60 **(B)** consolidating the data from both 0-14 and 0-28 schedules. Numbers above the bars show the Geometric Mean Titer (GMT), and the error bars indicate the 95% CI. A Wilcoxon test analyzed data to compare against the wild-type RBD; ****p < 0.0001. The graph represents the results obtained for 22 participants in the 18-59 years old group and 20 participants in the ≥60 years old group.

Supplementary Figure S2 | CoronaVac vaccination induces antibodies able to inhibit the interaction between hACE2 and S1-RBD from SARS-CoV-2 variants in participants aged 18-59 and ≥60 after two immunizations in both 0-14 and 0-28 schedules. Antibody titers were evaluated with a surrogate virus neutralization assay, which quantifies the interaction between S1-RBD from either Wild type SARS-CoV-2 or variants of concern (Alpha, Beta, Gamma and Delta) and hACE2 on ELISA plates. Results were obtained from participants vaccinated with CoronaVac 28 days after the second dose. For 0-14 schedule, volunteers between 18-59 and ≥ 60 are shown in **(A, B)**, respectively, and for 0-28, schedule volunteers between 18-59 and ≥ 60 are shown in **(C, D)**, respectively. The bars above show the Geometric Mean Titer (GMT), and the error bars indicate the 95% CI. A Wilcoxon test analyzed data to compare against the wild-type RBD; **p < 0.05, ***p < 0.005, ****p < 0.0001. The graph represents the results obtained for 12 participants in the 18-59 years old group and 10 participants in the ≥60 years old group in the 0-14 schedule and for 10 participants in the 18-59 years old group and 10 participants in the ≥60 years old group in the 0- 28 schedule.

Supplementary Figure S3 | CoronaVac immunization induces neutralizing antibodies against SARS-CoV-2 variants after two vaccine doses using a live virus test in volunteers aged 18-59 and over 60 years old. Antibody titers were evaluated by incubating the serum with a SARS-CoV-2 Chilean clinical strain and then added into Vero E6 cell for seven days. The neutralizing titer was determinate for the last dilution where no viralcytopathic effect was found in cells against wild type (D614G) and Alpha, Gamma and Delta variants. Consolidate neutralizing antibodies titer of volunteers from 0-14 and 0-28 schedules aged 18-59 years old are shown in **(A)**, while volunteer under 60 years old from 0-14 and 0-28 schedules are shown in **(B)**. The bars above show the Geometric Mean Titer (GMT), and the error bars indicate the 95% CI. A Wilcoxon test analysed data to compare against the wild-type RBD; *p < 0.05. The graph represents the results obtained for 42 volunteers of both schedules.

Supplementary Figure S4 | Evaluation of cellular immune response through ELISPOT upon stimulation with Mega Pools of Spike peptides derived from SARS-CoV-2 WT and variants of concern in volunteers immunized with CoronaVac. Numbers of IL-4-secreting cells, determined through ELISPOT as spot forming cells (SFCs) were determined. PBMCs were stimulated with MP-S WT, MP-S Alpha, MP-S Beta, MP-S Gamma and MP-Delta for 48 h for samples obtained 2 weeks after the second dose of volunteers of the 0-14 schedule (n = 11) and 0-28 schedule (n = 7). A total of 18 volunteers were evaluated. Data shown represents mean 95% CI and the mean is indicated above each bar. Statistical differences were evaluated by a one-way ANOVA followed by Dunnett's test for multiple comparisons against the MP-S WT.

REFERENCES

1. Dong E, Du H, Gardner L. An Interactive Web-Based Dashboard to Track COVID-19 in Real Time. *Lancet Infect Dis* (2020) 20:533–4. doi: 10.1016/S1473-3099(20)30120-1
2. Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, et al. SARS-CoV-2 Spike-Protein D614G Mutation Increases Virion Spike Density and Infectivity. *Nat Commun* (2020) 11:1–9. doi: 10.1038/s41467-020-19808-4
3. Ozono S, Zhang Y, Ode H, Sano K, Tan TS, Imai K, et al. SARS-CoV-2 D614G Spike Mutation Increases Entry Efficiency With Enhanced ACE2-Binding Affinity. *Nat Commun* (2021) 12:1–9. doi: 10.1038/s41467-021-21118-2
4. Jalkanen P, Kolehmainen P, Häkkinen HK, Huttunen M, Tähtinen PA, Lundberg R, et al. COVID-19 mRNA Vaccine Induced Antibody Responses and Neutralizing Antibodies Against Three SARS-CoV-2 Variants. *Nat Commun* (2021) 12:3991. doi: 10.21203/rs.3.rs-343388/v1
5. Daniloski Z, Jordan TX, Ilmain JK, Guo X, Bhabha G, tenOever BR, et al. The Spike D614g Mutation Increases Sars-Cov-2 Infection of Multiple Human Cell Types. *Elife* (2021) 10:e65365. doi: 10.7554/eLife.65365
6. Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, et al. SARS-CoV-2 Variants, Spike Mutations and Immune Escape. *Nat Rev Microbiol* (2021) 19:409–24. doi: 10.1038/s41579-021-00573-0
7. Liu Y, Liu J, Plante KS, Plante JA, Xie X, Zhang X, et al. The N501Y Spike Substitution Enhances SARS-CoV-2 Transmission. *bioRxiv Prepr Serv Biol* (2021), 2021.03.08.434499. doi: 10.1101/2021.03.08.434499
8. Chen RE, Zhang X, Case JB, Winkler ES, Liu Y, VanBlargan LA, et al. Resistance of SARS-CoV-2 Variants to Neutralization by Monoclonal and Serum-Derived Polyclonal Antibodies. *Nat Med* (2021) 27:717–26. doi: 10.1038/s41591-021-01294-w
9. Xie X, Liu Y, Liu J, Zhang X, Zou J, Fontes-Garfias CR, et al. Neutralization of SARS-CoV-2 Spike 69/70 Deletion, E484K and N501Y Variants by BNT162b2 Vaccine-Elicited Sera. *Nat Med* (2021) 27:620–1. doi: 10.1101/2021.01.27.427998
10. Ku Z, Xie X, Davidson E, Ye X, Su H, Menachery VD, et al. Molecular Determinants and Mechanism for Antibody Cocktail Preventing SARS-CoV-2 Escape. *Nat Commun* (2021) 12:4177. doi: 10.1038/s41467-021-24440-x
11. Campbell F, Archer B, Laurenson-Schafer H, Jinnai Y, Konings F, Batra N, et al. Increased Transmissibility and Global Spread of SARS-CoV-2 Variants of Concern as at June 2021. *Eurosurveillance* (2021) 26:2100509. doi: 10.2807/1560-7917.ES.2021.26.24.2100509
12. Saito A, Nasser H, Uriu K, Kosuge Y, Irie T, Shirakawa , et al. SARS-CoV-2 Spike P681R Mutation Enhances and Accelerates Viral Fusion. *bioRxiv* (2021) 2021.06.17.448820. doi: 10.1101/2021.06.17.448820
13. Deng X, Garcia-Knight MA, Khalid MM, Servellita V, Wang C, Morris MK, et al. Transmission, Infectivity, and Neutralization of a Spike L452R SARS-CoV-2 Variant. *Cell* (2021) 184:3426–37.e8. doi: 10.1016/j.cell.2021.04.025
14. Chilean Minister of Health: Ministerio de Salud Gobierno de Chile. Plan Nacional De Vacunación. Accessed at: https://www.minsal.cl/7052-de-la-poblacion-objetivo-ha-completado-su-vacunacion-contra-sars-cov-2/.
15. Bueno SM, Abarca K, González PA, Gálvez NMS, Soto JA, Duarte LF, et al. Safety and Immunogenicity of an Inactivated SARS-CoV-2 Vaccine in a Subgroup of Healthy Adults in Chile. *Clin Infect Dis* (2021) ciab823. doi: 10.1093/cid/ciab823/6372423
16. Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *N Engl J Med* (2021). doi: 10.1056/NEJMoa2107715
17. Valdespino Gómez JL, García García MDL. Declaración De Helsinki. *Gac Med Mex* (2001).

18. Tan CW, Chia WN, Qin X, Liu P, Chen MI, Tiu C, et al. A SARS-CoV-2 Surrogate Virus Neutralization Test Based on Antibody-Mediated Blockage of ACE2–spike Protein–Protein Interaction. *Nat Biotechnol* (2020). doi: 10.21203/rs.3.rs-24574/v1
19. Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, Tolerability, and Immunogenicity of an Inactivated SARS-CoV-2 Vaccine in Healthy Adults Aged 18–59 Years: A Randomised, Double-Blind, Placebo-Controlled, Phase 1/2 Clinical Trial. *Lancet Infect Dis* (2021). doi: 10.1016/S1473-3099(20)30843-4
20. Chen X, Chen Z, Azman AS, Sun R, Lu W, Zheng N, et al. Neutralizing Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Variants Induced by Natural Infection or Vaccination: A Systematic Review and Pooled Analysis. *Clin Infect Dis* (2021), ciab646. doi: 10.1093/cid/ciab646/6327511
21. Duarte LF, Gálvez NMS, Iturriaga C, Melo-González F, Soto JA, Schultz BM, et al. Immune Profile and Clinical Outcome of Breakthrough Cases After Vaccination With an Inactivated SARS-CoV-2 Vaccine. *Front Immunol* (2021) 12:742914/full. doi: 10.3389/fimmu.2021.742914/full
22. Barros-Martins J, Hammerschmidt SI, Cossmann A, Odak I, Stankov MV, Morillas Ramos G, et al. Immune Responses Against SARS-CoV-2 Variants After Heterologous and Homologous ChAdOx1 Ncov-19/BNT162b2 Vaccination. *Nat Med* (2021) 27:1525–29. doi: 10.1101/2021.06.01.21258172
23. Ranzani OT, Hitchings M, Dorion Nieto M, D'Agostini TL, de Paula RC, de Paula OFP, et al. Effectiveness of the CoronaVac Vaccine in the Elderly Population During a P.1 Variant-Associated Epidemic of COVID-19 in Brazil: A Test-Negative Case-Control Study. *MedRxiv* (2021) 374. doi: 10.1136/bmj.n2015
24. Wang K, Cao Y, Zhou Y, Wu J, Jia Z, Hu Y, et al. A Third Dose of Inactivated Vaccine Augments the Potency, Breadth, and Duration of Anamnestic Responses Against SARS-CoV-2. *medRxiv* (2021) 2021.09.02.21261735. doi: 10.1101/2021.09.02.21261735
25. Souza WM, Amorim MR, Sesti-Costa R, Coimbra LD, Brunetti NS, Toledo-Teixeira DA, et al. Neutralisation of SARS-CoV-2 Lineage P.1 by Antibodies Elicited Through Natural SARS-CoV-2 Infection or Vaccination With an Inactivated SARS-CoV-2 Vaccine: An Immunological Study. *Lancet Microbe* (2021) 2:e527–35. doi: 10.1016/S2666-5247(21)00129-4
26. Acevedo ML, Alonso-Palomares L, Bustamante A, Gaggero A, Paredes F, Cortés CP, et al. Infectivity and Immune Escape of the New SARS-CoV-2 Variant of Interest Lambda. *medRxiv* (2021) 2021.06.28.21259673. doi: 10.1101/2021.06.28.21259673
27. Fernández J, Bruneau N, Fasce R, Martín HS, Balanda M, Bustos P, et al. Neutralization of Alpha, Gamma, and D614G SARS-CoV-2 Variants by CoronaVac Vaccine-Induced Antibodies. *J Med Virol* (2021). doi: 10.1002/jmv.27310
28. Yuan M, Huang D, Lee CC, Wu NC, Jackson AM, Zhu X, et al. Structural and Functional Ramifications of Antigenic Drift in Recent SARS-CoV-2 Variants. *Sci (80-)* (2021) 373:818–23. doi: 10.1126/science.abh1139
29. Vacharathit V, Aiewsakun P, Manopwisedjaroen S, Srisaowakarn C, Laopanupong T, Ludowyke N, et al. CoronaVac Induces Lower Neutralising Activity Against Variants of Concern Than Natural Infection. *Lancet Infect Dis* (2021) 21:1352–4. doi: 10.1016/S1473-3099(21)00568-5
30. Liu Y, Liu J, Xia H, Zhang X, Fontes-Garfias CR, Swanson KA, et al. Neutralizing Activity of BNT162b2-Elicited Serum. *N Engl J Med* (2021) 384:1466–8. doi: 10.1056/NEJMc2102017
31. Liu J, Liu Y, Xia H, Zou J, Weaver SC, Swanson KA, et al. BNT162b2-Elicited Neutralization of B.1.617 and Other SARS-CoV-2 Variants. *Nature* (2021) 596:273–5. doi: 10.1038/s41586-021-03693-y
32. Planas D, Veyer D, Baidaliuk A, Staropoli I, Guivel-Benhassine F, Rajah MM, et al. Reduced Sensitivity of SARS-CoV-2 Variant Delta to Antibody Neutralization. *Nature* (2021) 596:276–80. doi: 10.1038/s41586-021-03777-9
33. Valenzuela MT, Urquidi C, Rodriguez N, Castillo L, Fernández J, Ramírez E. Development of Neutralizing Antibody Responses Against SARS-CoV-2 in COVID-19 Patients. *J Med Virol* (2021) 93:4334–41. doi: 10.1002/jmv.26939
34. Tarke A, Sidney J, Methot N, Yu ED, Zhang Y, Dan JM, et al. Impact of SARS-CoV-2 Variants on the Total CD4+ and CD8+ T Cell Reactivity in Infected or Vaccinated Individuals. *Cell Rep Med* (2021) 2:100355. doi: 10.1016/j.xcrm.2021.100355
35. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing Antibody Levels Are Highly Predictive of Immune Protection From Symptomatic SARS-CoV-2 Infection. *Nat Med* (2021) 27:1205–11. doi: 10.1038/s41591-021-01377-8

Conflict of Interest: Authors GZ and WM are employed by company SINOVAC Biotech. AS is a consultant for Gritstone Bio, Flow Pharma, Arcturus Therapeutics, ImmunoScape, CellCarta, Avalia, Moderna, Fortress and Repertoire.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

La Jolla Institute for Immunology (LJI) has filed for patent protection for various aspects of T cell epitope and vaccine design work.

The authors declare this study received the investigational product (placebo and vaccines) from the company SINOVAC Biotech. SINOVAC employees contributed to the conceptualization of the study (clinical protocol and eCRF design) but did not participate in either the analysis or interpretation of the data shown in this manuscript.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Immunology

ORIGINAL RESEARCH
published: 29 September 2021
doi: 10.3389/fimmu.2021.742914

Immune Profile and Clinical Outcome of Breakthrough Cases After Vaccination With an Inactivated SARS-CoV-2 Vaccine

Luisa F. Duarte[1,2†], Nicolás M. S. Gálvez[1,2†], Carolina Iturriaga[3†], Felipe Melo-González[1,2†], Jorge A. Soto[1,2†], Bárbara M. Schultz[1,2†], Marcela Urzúa[3†], Liliana A. González[1,2], Yaneisi Vázquez[1,2], Mariana Ríos[1,2], Roslye V. Berríos-Rojas[1,2], Daniela Rivera-Pérez[1,2], Daniela Moreno-Tapia[1,2], Gaspar A. Pacheco[1,2], Omar P. Vallejos[1,2], Guillermo Hoppe-Elsholz[1,2], María S. Navarrete[4], Álvaro Rojas[4], Rodrigo A. Fasce[5], Jorge Fernández[5], Judith Mora[5], Eugenio Ramírez[5], Gang Zeng[6], Weining Meng[6], José V. González-Aramundiz[7], Pablo A. González[1,2], Katia Abarca[1,3], Susan M. Bueno[1,2*] and Alexis M. Kalergis[1,2,8*]*

[1] Millennium Institute on Immunology and Immunotherapy, Santiago, Chile, [2] Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile, [3] Departamento de Enfermedades Infecciosas e Inmunología Pediátricas, División de Pediatría, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile, [4] Departamento de Enfermedades Infecciosas del Adulto, División de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile, [5] Departamento de Laboratorio Biomédico, Instituto de Salud Pública de Chile, Santiago, Chile, [6] Sinovac Biotech, Beijing, China, [7] Departamento de Farmacia, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile, [8] Departamento de Endocrinología, Facultad de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

OPEN ACCESS

Edited by:
Rong Hai,
University of California, Riverside, United States

Reviewed by:
Daniel Ramos Ram, Beth Israel Deaconess Medical Center and Harvard Medical School, United States
Duo Xu, University of California, Riverside, United States

***Correspondence:**
Katia Abarca
kabarca@uc.cl
Susan M. Bueno
sbueno@bio.puc.cl
Alexis M. Kalergis
akalergis@bio.puc.cl

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Vaccines and Molecular Therapeutics, a section of the journal Frontiers in Immunology

Received: *16 July 2021*
Accepted: *06 September 2021*
Published: *29 September 2021*

Citation:
Duarte LF, Gálvez NMS, Iturriaga C, Melo-González F, Soto JA, Schultz BM, Urzúa M, González LA, Vázquez Y, Rios M, Berríos-Rojas RV, Rivera-Pérez D, Moreno-Tapia D, Pacheco GA, Vallejos OP, Hoppe-Elsholz G, Navarrete MS, Rojas Á, Fasce RA, Fernández J, Mora J, Ramírez E, Zeng G, Meng W, González-Aramundiz JV, González PA, Abarca K, Bueno SM and Kalergis AM (2021) Immune Profile and Clinical Outcome of Breakthrough Cases After Vaccination With an Inactivated SARS-CoV-2 Vaccine. Front. Immunol. 12:742914. doi: 10.3389/fimmu.2021.742914

Constant efforts to prevent infections by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are actively carried out around the world. Several vaccines are currently approved for emergency use in the population, while ongoing studies continue to provide information on their safety and effectiveness. CoronaVac is an inactivated SARS-CoV-2 vaccine with a good safety and immunogenicity profile as seen in phase 1, 2, and 3 clinical trials around the world, with an effectiveness of 65.9% for symptomatic cases. Although vaccination reduces the risk of disease, infections can still occur during or after completion of the vaccination schedule (breakthrough cases). This report describes the clinical and immunological profile of vaccine breakthrough cases reported in a clinical trial in progress in Chile that is evaluating the safety, immunogenicity, and efficacy of two vaccination schedules of CoronaVac (clinicaltrials.gov NCT04651790). Out of the 2,263 fully vaccinated subjects, at end of June 2021, 45 have reported symptomatic SARS-CoV-2 infection 14 or more days after the second dose (1.99% of fully vaccinated subjects). Of the 45 breakthrough cases, 96% developed mild disease; one case developed a moderate disease; and one developed a severe disease and required mechanical ventilation. Both cases that developed moderate and severe disease were adults over 60 years old and presented comorbidities. The immune response before and after SARS-CoV-2 infection was analyzed in nine vaccine breakthrough cases, revealing that six of them exhibited circulating anti-S1-RBD IgG antibodies with neutralizing capacities

after immunization, which showed a significant increase 2 and 4 weeks after symptoms onset. Two cases exhibited low circulating anti-S1-RBD IgG and almost non-existing neutralizing capacity after either vaccination or infection, although they developed a mild disease. An increase in the number of interferon-γ-secreting T cells specific for SARS-CoV-2 was detected 2 weeks after the second dose in seven cases and after symptoms onset. In conclusion, breakthrough cases were mostly mild and did not necessarily correlate with a lack of vaccine-induced immunity, suggesting that other factors, to be defined in future studies, could lead to symptomatic infection after vaccination with CoronaVac.

Keywords: CoronaVac, phase 3 clinical trial, SARS-CoV-2, COVID-19, vaccines, breakthrough cases

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus first identified in China, in December of 2019, and is responsible of the current worldwide pandemic with nearly 4 million deaths reported at the beginning of July 2021 (1, 2). Coronavirus disease 2019 (COVID-19) is the result of infection caused by this virus, a disease that ranges from mild respiratory symptoms in over 80% of the population to severe illnesses requiring oxygen assistance and invasive ventilation, which usually leads to fatal or life-threatening outcomes (3).

Vaccine development has become the main hope for reducing COVID-19 cases and the severity of this disease (4). Several vaccines have been developed through different molecular approaches (i.e., viral mRNA, viral recombinant proteins, recombinant viral vectors, or inactivated whole virus), and up to date, the World Health Organization (WHO) has granted emergency approval for the use of 10 of them (5). Despite their differences, all these vaccines have reported a protective immune response against SARS-CoV-2 infections in clinical trials (6). Several studies have reported the production of antibodies with neutralizing capacities, along with broad cellular immune responses that helps in the clearance of the virus (6–10). However, breakthrough cases, defined as the detection of SARS-CoV-2 RNA in people ≥14 days after they completed the immunization schedule, have been reported (11, 12). These cases push the scientific community towards a further characterization and comprehension of the immune response elicited upon vaccination, in order to achieve enhanced protective responses in all the population.

CoronaVac is an inactivated SARS-CoV-2 vaccine that has shown to be 65.9%, 87.5%, 90.3%, and 86.3% effective in preventing COVID-19 symptoms, hospitalization, ICU admission, and COVID-19-related death, respectively, as recently reported in a cohort of almost 10.2 million individuals in Chile (13). It has been reported that immunization with CoronaVac elicits an immune response directed against several viral components, beyond the spike (S) protein, after the administration of two doses, as evidenced by detecting IgG antibodies against N protein and a substantial $CD4^+$ T-cell response after *ex vivo* stimulation with a MegaPool (MP) of peptides covering the remainder "non-spike" SARS-CoV-2 proteome (7, 14, 15). Phase 3 clinical trials for this vaccine are being held in different countries around the globe (15, 16). Particularly in Chile, a clinical trial is undergoing to evaluate two different immunization schedules, with the second dose administered either 2 (0–14) or 4 (0–28) weeks after the first one (clinicaltrials.gov number: NCT04651790). Among 2,263 fully vaccinated volunteers, on June 25, 2021, a total of 45 COVID-19 cases (1.99%) have been reported occurring in the monitoring period (from 2 weeks after the second dose). Here, we report the clinical outcome and the immune response elicited by nine breakthrough cases detected among the 15 of the 450 volunteers enrolled in the immunogenicity branch of the phase 3 clinical trial, who already received both doses of CoronaVac. Evaluation of the humoral immune response considered the measurement of circulating anti-S1-RBD IgG antibodies and their neutralizing capacities as measured by two different techniques. Evaluation of the cellular immune response was performed through ELISPOT assays after *ex vivo* stimulation of peripheral blood mononuclear cells (PBMCs) with two sets of MP of peptides derived from the proteome of SARS-CoV-2 (17). A thorough understanding of the immune responses elicited after vaccination and as to how it correlates with the protection elicited after this and subsequent infections will provide valuable information that will improve the approaches currently being used to halt the COVID-19 pandemic and will also indicate whether an additional dose of currently approved vaccines is needed after a certain time span.

MATERIALS AND METHODS

Study Design, Volunteers, and Randomization

The clinical trial (clinicaltrials.gov NCT04651790) was conducted in Chile at eight different sites and evaluated two immunization schedules in a 1:1 ratio. This trial was approved by each Institutional Ethical Committee and by the Chilean Public Health Institute (#24204/20) and conducted according to the current Tripartite Guidelines for Good Clinical Practices, the Declaration of Helsinki (18), and local regulations. Written informed consent was obtained from each participant. Volunteers included men and women aged ≥18, inoculated with two doses of 3 μg (600SU) of CoronaVac. One group received the second dose 2 weeks after the first dose (0–14 schedule), while a second group received the second dose 4 weeks after the first one (0–28 schedule). Exclusion criteria included, among others, history

of confirmed symptomatic SARS-CoV-2 infection, pregnancy, allergy to vaccine components, and immunocompromised conditions. A complete list of inclusion and exclusion criteria has been published previously (15).

A total of 2,302 volunteers were enrolled by March 19, 2021, of whom 2,263 received both doses. A subgroup of 450 volunteers was selected to evaluate their immune response, receiving randomly CoronaVac either in a 0–14 or a 0–28 immunization schedule (1:1 ratio). Demographic information, comorbidities, nutritional status, immunization schedule, and dates of vaccination were obtained at enrollment and registered in the electronic case-report form (eCRF) for all volunteers. Nutritional status was determined using a gender and body mass index (BMI) (19).

Breakthrough Case Follow-Up

Confirmed COVID-19 cases reported 14 days after the administration of the second dose of CoronaVac were identified following the protocol procedures for efficacy. Briefly, upon enrollment, participants were instructed to report through an electronic platform, e-mail, cell phone message, or telephone call, each time the definition for suspected positive case was met. A positive case was suspected if at least one of the following symptoms were present for over 2 days: fever or chills, coughing, shortness of breath or breathing difficulty, fatigue, muscle or body pain, headache, loss of smell or taste, sore throat, nasal congestion or runny nose, nausea or vomiting, and diarrhea. Upon the report, an evaluation visit was scheduled with a study physician, for 3 days after symptoms onset, to evaluate the presence of SARS-CoV-2 RNA by reverse-transcriptase quantitative PCR (RT-qPCR) in nasopharyngeal (NP) sample. If the sample was negative, and at least one symptom persisted, a second test was performed after 48 h. If a sample was positive, the clinical evolution of the case was closely monitored by the center personnel until its resolution. If hospitalization was required, information was obtained from relatives of the volunteer and from clinical reports.

Upon confirmation of positive cases, history of possible close contact with confirmed COVID-19 cases and the severity and duration of each signs and symptoms were registered. Severity was classified from grades 1 to 4, as published previously by the Food and Drug Administration (FDA) and the National Institutes of Health (NIH) (20, 21). Intensity of the disease was graded from score 1 to 9, as published previously by the WHO (22). The grading for severity criteria indicated in the protocol were either mild (symptomatic patients without viral pneumonia or hypoxia), moderate (clinical signs of pneumonia such as fever, coughing, shortness of breath, difficulty breathing but no signs of severe pneumonia, oxygen saturation ≥94% on room air), or severe {resting clinical signs indicative of severe clinical illness [respiratory rate (RR) ≥30/min; heart rate (HR) ≥125/min; oxygen saturation <94% at room air at sea level; PaO_2/FiO_2 <300 mm Hg], respiratory failure [requirement of high-flow oxygen, noninvasive ventilation, mechanical ventilation, or extracorporeal membrane oxygenation (ECMO)], evidence of shock [systolic blood pressure (SBP) <90 mmHg, diastolic blood pressure (DBP) <60 mmHg, or requirement of vasopressors], significant acute renal, hepatic, or neurological dysfunction, admission to ICU, or death}. All this information was recorded in both the clinical file of the participant and the eCRF.

Procedures

To evaluate the immune response elicited upon immunization, peripheral blood samples were obtained for the isolation of serum and PBMCs. For volunteers from the immunogenicity branch, samples were collected before the first and the second dose and 2 and 4 weeks after the second dose. After COVID-19 confirmation by PCR, two additional peripheral blood samples were obtained about 2 and 4 weeks after symptoms onset (follow-up 1 and 2, respectively). Sera samples and PBMC were collected as previously reported (15) and stored at −80°C or in liquid nitrogen, respectively.

Circulating IgG antibodies specific against the RBD of the S1 protein of SARS-CoV-2 (S1-RBD) were measured using the COVID-19 Human Antibody Detection Kit (RayBio #IEQ-CoVS1RBD-IgG), following the instructions of the manufacturer. Sera samples were two-fold serially diluted, starting at a 200-fold dilution until a 6,400-fold dilution. The antibody titer was determined as the last fold dilution with an absorbance over the cut-off value. The cut-off value for each dilution was determined as 2.1 times the absorbance at 450 nm for a panel of 29 seronegative samples.

The neutralizing capacities of circulating antibodies were determined by two different techniques, i.e., through a surrogate virus neutralizing test (sVNT) and a conventional plaque-reduction neutralization test (cVNT). The sVNT were performed following the instructions of the manufacturer (BioHermes #COV-S41), and sera samples were 2-fold serially diluted starting at a 4-fold dilution until a 4,096-fold dilution. The percentage of inhibition was defined as follows: ($OD_{450\ nm}$ value of negative control − $OD_{450\ nm}$ value of sample)/($OD_{450\ nm}$ value of negative control × 100), and titers were reported as the reciprocal of the highest serum dilution required to achieve 30% of inhibition. Samples exhibiting <30% inhibitory activity at the lowest dilution tested (1:4) were assigned a titer of 2. For the cVNT, sera samples were 2-fold serially diluted starting at a 4-fold dilution until a 512-fold dilution. Then, samples were incubated with a SARS-CoV-2 clinical isolate (33782CL-SARS-CoV-2 strain) for 1 h at 37°C. The mixtures were then added to Vero E6 cell monolayers (ATCC CRL-1586), and cytopathic effect (CPE) was evaluated 7 days after infection. Positive and negative controls were held for each assay. CPE was evaluated by direct visualization, and the titer of neutralizing antibodies was defined as the latest fold dilution exhibiting 100% of infection inhibition and absence of CPE. A titer of 2 was assigned for samples showing CPE at the lowest dilution tested (1:4).

The cellular immune response was evaluated through ELISPOT assays, as described previously, using the human interferon (IFN)-γ/IL-4 double-color ELISPOT (Immunospot) (15). Cells were cultured for 48 h in the presence of four different SARS-CoV-2-specific MPs (17). Two of these MPs are composed of 15-mer peptides derived from the S protein (MP-S) and the remaining proteins of the viral particle (MP-R). The other two MPs are composed of 9- to 11-mer peptides from the whole proteome of SARS-CoV-2 (CD8-A and CD8-B). Positives and

negative controls were considered for each assay as reported previously (15, 17).

RESULTS

Clinical Features of Breakthrough Cases

From January 1 to June 25, 2021, 50 breakthrough cases were reported among the 2,263 vaccinated volunteers that had received two vaccine doses, of which 45 had over 14 days after the second dose (26 cases in the 0–14 schedule and 19 in the 0–28 schedule). Fifteen of these breakthrough cases were among the 450 volunteers in the immunogenicity branch. Eight of these had follow-up samples from days 14 and 30 after the start of symptoms of COVID-19, and one of them had a single follow-up sample taken 14 days after symptoms onset (Volunteer 1). All nine were Hispanic–Latin and were negative for the presence of circulating S- and N-SARS-CoV-2 IgG antibodies at recruitment. Six of them received the 0–14 immunization schedule and three the 0–28 immunization schedule (**Figure 1**). The demographic characteristics and relevant clinical history of cases are shown in **Table 1**.

Intensity and severity of the disease were mild, with a score of 2 in seven out of the nine cases (Volunteers 1, 2, 3, 5, 6, 8, and 9), and the symptoms exhibited by them in decreasing frequency were nasal congestion (seven cases), sore throat (six), loss of smell (six), headache (five), coughing (four), loss of taste (four), runny nose (four), fatigue or myalgia (three), dyspnea (one), nausea (one), and diarrhea (one). None of the seven cases exhibited fever or vomiting. Accordingly, the duration of each symptoms was nasal congestion (1–13 days), sore throat (1–12), loss of smell (3–10), headache (5–13), cough (1–8), loss of taste (3–10), runny nose (2–13), fatigue (4–12), myalgia (1–21), dyspnea (12), nausea (4), and diarrhea (4–5). Most of the symptoms recorded were grade 1 or 2. The clinical outcome of the COVID-19 disease for each volunteer is indicated in **Table 2**.

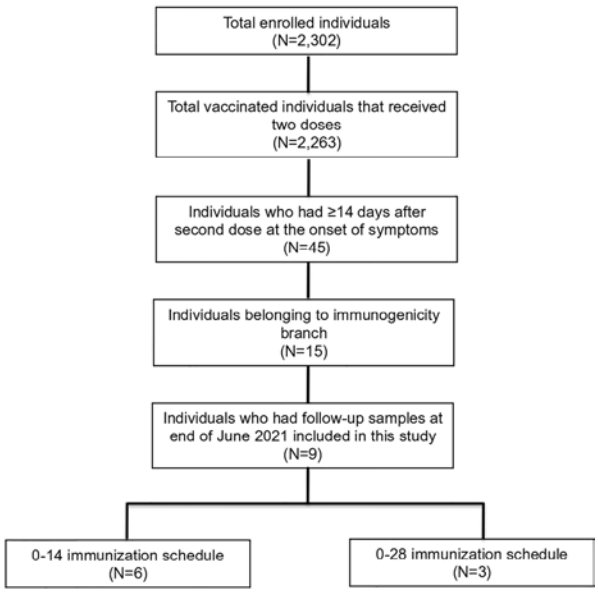


FIGURE 1 | Enrolled volunteers and breakthrough cohort included in this study. Nine of the 2,302 vaccinated individuals belonging to the clinical trial conducted in Chile were included in this study after confirming COVID-19 disease by reverse-transcriptase polymerase chain-reaction (RT-qPCR) assay. They were selected from 45 individuals who displayed symptoms after ≥14 days from the administration of the second dose of the vaccine because they were enrolled in the immunogenicity branch and further had at least one follow-up sample after symptoms onset at the end of June of 2021.

Two out of the nine breakthrough cases (Volunteers 4 and 7) reached a score over 2. The highest clinical score registered for Volunteers 4 was 5 (moderate), and for Volunteer 7 was 7 (severe). Volunteer 4 is a 62-year-old man, with a BMI of 29.3 (overweight) and is currently being treated for hypothyroidism (**Table 1**). The onset date was 122 days after the administration of the second dose (0–28 immunization schedule), and no close contact with a COVID-19-positive case was reported. The symptoms exhibited were fatigue, muscle pain, headache, nasal congestion, cough, and fever. After 6 days of disease development, Volunteer 4 was hospitalized due to persistent symptoms and the addition of shortness of breath to the list. A chest CT confirmed COVID-19 pneumonia. He was diagnosed with acute respiratory insufficiency and then received 4 L/min of oxygen by nasal cannula for 4 days. After this, he exhibited an overall improvement and recovery, with a total time of hospitalization of 8 days. Volunteer 7 is a 69-year-old man, with a BMI of 28.0 (overweight) and a history of arterial hypertension, bicuspid aorta, and atrial fibrillation. The onset date was 32 days after the administration of the second dose (0–28 immunization schedule), and close contact with a COVID-19-positive case was confirmed (his son). He presented respiratory symptoms and fever. Later, onset and persistence of malaise and fever, the onset of dyspnea, and the confirmation of COVID-19 pneumonia by a chest CT led to hospitalization. All the typical COVID-19 symptoms except nausea, vomiting, and diarrhea were reported after hospitalization. He received supplemental oxygen by nasal cannula and was transferred to ICU due to heart failure. He required mechanical ventilation for 6 days and eventually recovered, with a total time of hospitalization of 20 days.

Remarkably, as described below, two out of the nine breakthrough cases (Volunteers 2 and 6) exhibited a weak immune response upon immunization and infection. Volunteer 2 is a 48-year-old man, with a BMI of 28.9 (overweight) and a history of hypothyroidism, arterial hypertension, coronary heart disease (acute myocardial infarction on September 2020), fatty liver disease, and dyslipidemia under treatment. During his childhood, he was diagnosed with influenza-associated encephalitis (4 years old, hospitalized in ICU) and with uncomplicated diphtheria (6 years old). During his adulthood, he was diagnosed with a post-influenza pneumonia in 2000 and with a clinically suspected *Mycoplasma pneumonia* infection in 2018, both were treated with oral antibiotics. The symptoms onset was 26 days after the administration of the second dose (0–14 immunization schedule), and no contact with a COVID-19-

TABLE 1 | Demographic and clinical history of nine vaccine breakthrough cases.

Volunteer	Biological Sex*	Age	Nutritional Status	BMI	Co-morbidities
1	F	46	Normal	23.2	Migraine syndrome, allergic rhinitis
2	M	48	Overweight	28.9	Arterial hypertension, coronary heart disease, hypothyroidism
3	F	24	Overweight	25.3	Allergic rhinitis, penicillin allergy
4	M	62	Overweight	29.3	Hypothyroidism
5	F	32	Normal	23.9	Allergic rhinitis
6	F	33	Normal	20.5	Hypothyroidism
7	M	69	Overweight	28.0	Arterial hypertension, bicuspid aorta, atrial fibrillation, nephrolitiasis
8	F	28	Overweight	27.3	None
9	F	59	G2 Obesity	36.4	Insulin resistance

**Gray shading, female; no shading, male.*

TABLE 2 | Clinical development of COVID-19 disease in the nine breakthrough cases described.

Volunteer*	Immunization schedule	Day of symptoms onset^	Possible close contact with COVID-19 case	Required Hospitalization	Highest clinical score
1	0–14	37	Yes	No	2
2	0–14	23	No	No	2
3	0–14	43	No	No	2
4	0–14	122	No	Yes	5
5	0–14	122	No	No	2
6	0–14	94	No	No	2
7	0–28	32	Yes	Yes	7
8	0–28	34	No	No	2
9	0–28	16	Yes	No	2

**Gray shading, female; no shading, male.*
^Days after the administration of the second dose.

positive case was reported. He presented fatigue, headache, nasal congestion, runny nose, coughing, and diarrhea. Volunteer 6 is a 33-year-old woman, with a BMI of 20.5 (eutrophic), and medical history of mononucleosis (2003), recurrent herpes simplex labialis (since 2003), hypothyroidism, and currently on oral contraceptive therapy. No contact with a COVID-19-positive case was reported, and the onset date was 94 days after the administration of the second dose (0–14 immunization schedule). She presented fatigue, muscular pain, loss of smell, loss of taste, sore throat, and nasal congestion.

Altogether, the immunization schedule, medical history, demographic characteristics, the symptoms onset day, reporting of close contact with COVID-19 confirmed cases, and the symptoms exhibited by all breakthrough cases are diverse, and an evident pattern of conditions leading to susceptibility towards SARS-CoV-2 infection is not observed.

Humoral Immunity in Breakthrough Cases

To evaluate the humoral immune response elicited by the nine breakthrough cases, circulating IgG antibodies specific against the S1-RBD of SARS-CoV-2 were evaluated as indicated in *Materials and Methods*. As shown in **Figure 2** (and individually for each volunteer in **Supplementary Figure S1**), three out of the six cases from the 0-14 immunization schedule (Volunteers 1, 3, and 5) exhibited detectable levels of IgG antibodies specific against the S1-RBD at 4 weeks after the administration of the second dose (**Figure 2A** and **Supplementary Figures S1A, C, E**). This was also found for all three subjects in the 0–28 immunization schedule, although Volunteer 7 showed a weak response (**Figure 2B** and **Supplementary Figures S1G–I**). Circulating antibodies specific against S1-RBD also increased drastically 2 and 4 weeks after disease onset for all volunteers, except for Volunteers 2 and 6, that exhibited no changes in their antibodies profile throughout the time points evaluated.

The neutralizing capacities of the circulating antibodies measured in these nine breakthrough cases were also evaluated by two different techniques, as indicated in *Materials and Methods*. As evaluated by sVNT, five out of six cases in the 0–14 immunization schedule exhibited detectable levels of neutralizing antibodies 4 weeks after the administration of the second dose (**Figure 3A** and **Supplementary Figures S2A–F**). As expected, Volunteers 2 and 6 exhibited a very weak neutralizing capacity at this time point evaluated. However, upon evaluation by cVNT, only three volunteers in the 0–14 immunization schedule (Volunteers 1, 3, and 5) showed detectable neutralizing response (**Figure 3C**), which is in line

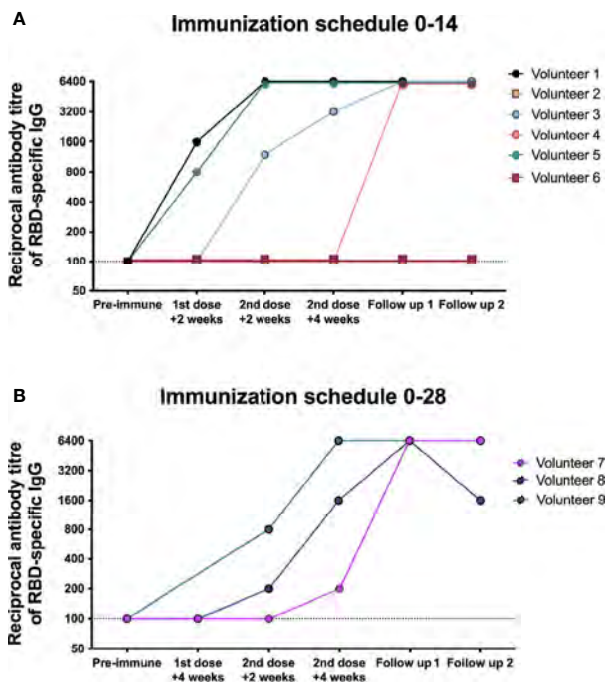


FIGURE 2 | Circulating antibodies response elicited in the nine breakthrough cases measured as IgG specific against the S1-RBD of SARS-CoV-2. Specific IgG antibodies against the S1-RBD of SARS-CoV-2 were evaluated in nine breakthrough cases that received two doses of CoronaVac. The figure shows the antibody titer in the serum samples obtained before administration of the first dose (pre-immune), before administration of the second dose (1st dose + 2 weeks or 1st dose + 4 weeks), 2 and 4 weeks after the second dose, and 2 and 4 weeks after the disease onset and a confirmed PCR result for SARS-CoV-2 (follow-up 1 and 2, respectively) and a confirmed PCR result for SARS-CoV-2. **(A)** shows the six volunteers enrolled in the 0–14 immunization schedule, and **(B)** shows the three volunteers enrolled in the 0–28 immunization schedule.

with the results obtained for IgG antibodies specific against the S1-RBD (**Figure 2A**). Notably, no neutralizing capacities were detected for the antibodies of Volunteer 4 (who displayed a moderate disease development) 2 or 4 weeks after the second dose, for both sVNT and cVNT (**Figures 3A, C**). All three cases in the 0–28 immunization schedule had detectable levels of neutralizing antibodies, by both sVNT and cVNT, 2 and 4 weeks after the administration of the second dose (**Figures 3B, D**). Noteworthy, Volunteer 7 (who developed severe symptoms) exhibited a very weak neutralizing capacity at these time points evaluated. As also seen for the circulating IgG antibodies specific against the S1-RBD, the neutralizing capacities of most volunteers increased drastically 2 and 4 weeks after the onset of disease symptoms, even for Volunteer 4, who exhibited no response after vaccination (**Figures 3A–D**).

IFN-γ Releasing by T Cells in Breakthrough Cases

To evaluate the cellular immune response elicited in these nine breakthrough cases, ELISPOT assays were performed as seen on **Figure 4** and **Supplementary Figure S3**. The number of spot-forming cells (SFC) positive for IFN-γ upon stimulation with MPs of peptides derived from SARS-CoV-2 were measured, as described in *Materials and Methods*. For most volunteers, upon stimulations with MPs containing 15-mer peptides (MP-S and MP-non-spike), SFC values measured in samples obtained 2 weeks after the administration of the second dose exhibited at least a two-fold increase as compared to those obtained before the administration of the first dose (**Figure 4A** for the 0–14 immunization schedule and **Figure 4B** for the 0–28 immunization schedule). Interestingly, Volunteer 6 showed no remarkable changes in the SFC values up to 4 weeks after the second dose, similar to that observed for Volunteer 9. SFC values increased for all volunteers (except Volunteer 2) 2 or 4 weeks after disease onset. Overall, SFC values obtained were higher when stimulating with MPs containing 15-mer peptides compared to those obtained when stimulating with MPs containing 9- to11-mer peptides (MP-CD8A and B) for both immunization schedules (**Figures 4A, C** for the 0–14 immunization schedule and **Figures 4B, D** for the 0–28 immunization schedule). Remarkably, Volunteer 6 displayed a good cellular response both after vaccination and infection, despite exhibiting a poor humoral response. The variation in SFC values for each volunteer after stimulation of MP-S and MP-non-spike and MP-CD8A and B is shown in **Supplementary Figure S3** and **Supplementary Tables 1**, **2**.

Overall, the results suggest that the cellular immune response elicited after either vaccination or infection in these nine breakthrough cases does not necessarily correlate with protection against SARS-CoV-2.

Immune Responses of Vaccine Breakthrough Cases as Compared to a Control Cohort

For the purpose of better understanding whether the immune response elicited after vaccination in breakthrough cases was an exclusive feature and a determining factor in the susceptibility to the further infection, we compared the humoral and cellular-mediated immune response of breakthrough cases with the response observed in a control group of individuals vaccinated with similar characteristics to the breakthrough population, but without manifestation of clinical symptoms related to COVID-19. Control cohort consisted of 18 subjects who received two doses of CoronaVac on similar dates to the breakthrough cases and shared demographic characteristics as detailed in **Supplementary Table 3**.

As observed in **Figure 5A**, breakthrough cases show neutralizing antibodies titers about two-fold lower than the control group for sVNT, with geometric mean titers (GMTs) of 9.5 (95% CI, 3.1–28.7) vs. 31 (95% CI, 17.8–53.2) and 13.7 (95% CI, 4.5–42.2) vs. 24 (95% CI, 14.2–38.9), 2 and 4 weeks after the second dose, respectively. In a similar way, the GMTs in the breakthrough group were approximately four-fold lower than those obtained by the control cohort for cVNT, 4.5 (95% CI, 2–10) vs. 18.7 (95% CI, 8.8–39.6) and 5.4 (95% CI, 2.5–11.6) vs. 28.5 (95% CI, 15–54.6), 2 and 4 weeks after the second dose, respectively. Importantly, these trends were sustained when titers of neutralizing antibodies from six additional breakthrough cases, which had data available for samples after vaccination, were added to the analysis (**Supplementary Figure S4**).

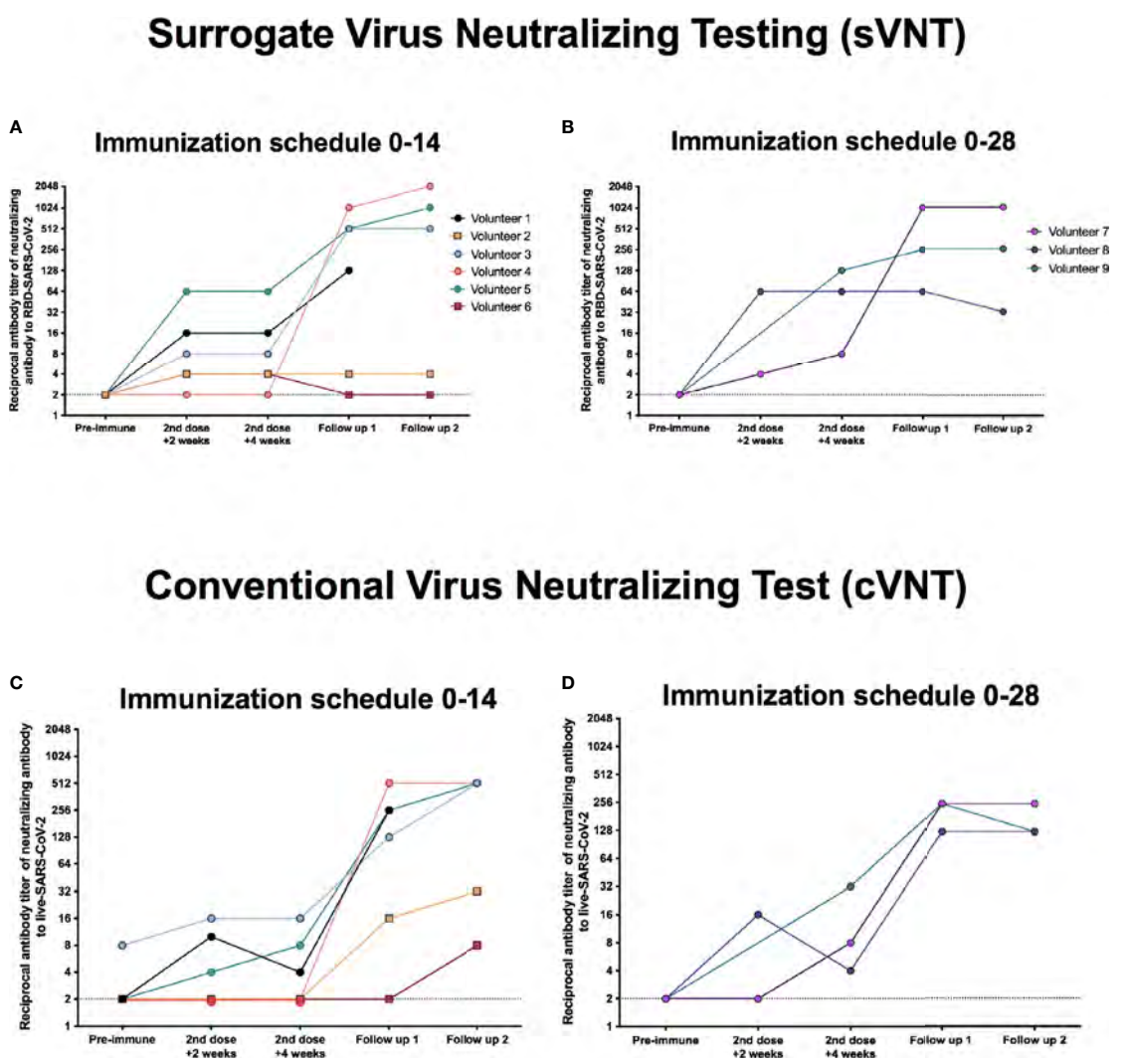


FIGURE 3 | Circulating antibodies exhibit varying neutralizing capacities in the nine breakthrough cases. Neutralizing antibodies were evaluated before administration of the first dose (pre-immune), 2 and 4 weeks after the second dose, and 2 and 4 weeks after the disease onset (follow-up 1 and 2, respectively). Two different techniques were used, a surrogate virus neutralization test (sVNT) based on the perturbation of the hACE2-spike protein–protein interaction mediated by antibodies, and a conventional virus neutralization test (cVNT) evaluating plaque and CPE reduction. **(A)** Neutralizing antibody titers detected by using the sVNT in six volunteers enrolled in the 0–14 immunization schedule. **(B)** Neutralizing antibody titers detected by using the sVNT in three volunteers enrolled in the 0–28 immunization schedule. **(C)** Neutralizing antibody titers detected by using the cVNT in six volunteers enrolled in the 0–14 immunization schedule. **(D)** Neutralizing antibody titers detected by using the cVNT in three volunteers enrolled in the 0–28 immunization schedule.

Conversely, we observed a better cellular response after stimulation with 15-mer MPs in the breakthrough cases than the control group at 2 weeks after the second dose administration, which decreased at 4 weeks after the second dose to lower levels than the control group. Regarding the 9- to 11-mer MPs stimulating (mainly $CD8^+$ T cells), a greater response was observed in the control group but only in approximately 50% of the individuals at 4 weeks after the second dose (**Figure 5B**).

In summary, these results show that detection of low levels of neutralizing antibodies after vaccination could be related to symptomatic infection; however, unknown underlying conditions must be affecting this susceptibility because low titers were also observed in some individuals belonging to the control group and high titers in the breakthrough group.

DISCUSSION

The use of different vaccines approved for emergency use due to the rapid spread of SARS-CoV2 has been key in stopping the uncontrolled progression of deaths worldwide. However, it has

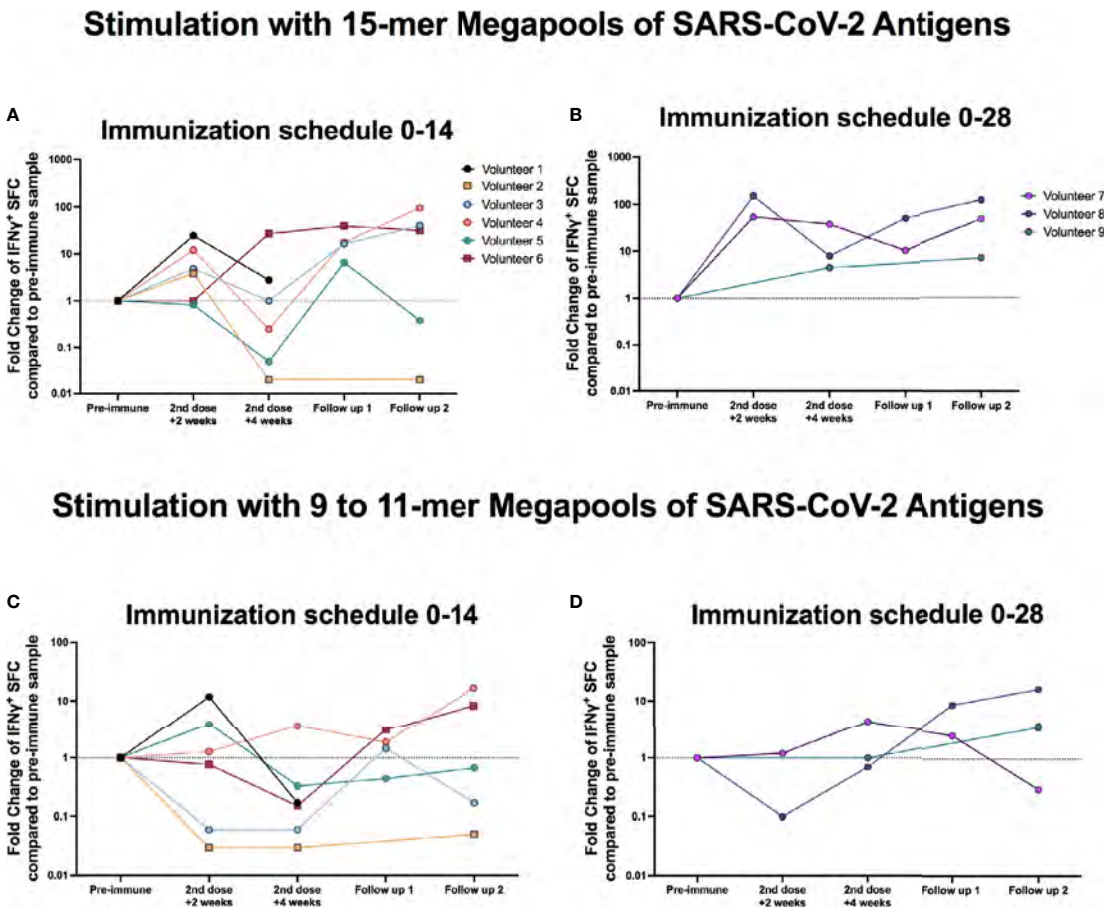


FIGURE 4 | The IFN-γ production by T cells from breakthrough cases after stimulation with MegaPools of SARS-CoV-2 peptides is heterogeneous. PBMCs from the nine breakthrough cases were obtained before administration of the first dose (pre-immune), 2 and 4 weeks after the second dose, and 2 and 4 weeks after the disease onset (follow-up 1 and 2, respectively) and evaluated by ELISPOT assays. Cells were stimulated for 48 h with two MPs containing several peptides from SARS-CoV-2 to induce the secretion IFN-γ by T cells. The number of spots-forming cells (SFCs) was evaluated. Data are shown as the fold increase regarding to the preimmune value for SFCs. **(A)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 15-mer peptides from SARS-CoV-2 of six volunteers enrolled at the 0–14 immunization schedule. **(B)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 15-mer peptides from SARS-CoV-2 of three volunteers enrolled at the 0–28 immunization schedule. **(C)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 9- to 11-mer peptides from SARS-CoV-2 of six volunteers enrolled at the 0–14 immunization schedule. **(D)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 9- to 11-mer peptides from SARS-CoV-2 of three volunteers enrolled at the 0–28 immunization schedule.

been reported that people with comorbidities can develop a more severe disease upon infection with SARS-CoV-2 (23). In this line, the efficacy of these vaccines can be impaired by the existence of previously described diseases or pathologies (24). In addition, the severity of the disease can be even more pronounced in the elderly, as they exhibit higher dysfunction in their immune system as compared to young people (25).

In this clinical trial, a total of 2,263 volunteers were vaccinated with two doses in two different immunization schedules. Out of all these volunteers, a total of 450 were part of the immunogenicity profile evaluation group. Here, we report the clinical outcome and immune response elicited by nine volunteers from the immunogenicity branch that were infected with SARS-CoV-2 and developed mild, moderate, or severe cases of COVID-19. Our results showed that the humoral and cellular immune response elicited by breakthrough CoronaVac cases was heterogeneous, and at least in these nine individuals, a correlate of infection was not evident. Yet, older people have a greater susceptibility to develop severe diseases as compared to younger people.

Of these nine volunteers, six exhibited some degree of overweight, and only one volunteer did not have any comorbidity. Two volunteers developed diseases that required hospitalization. Volunteer 7, a 69-year-old man, reported four comorbidities and required mechanical ventilation. Volunteer 4, a 62-year-old man, reported two comorbidities and required supplemental oxygen. Remarkably, in line with the results shown here, various publications have suggested that men are more

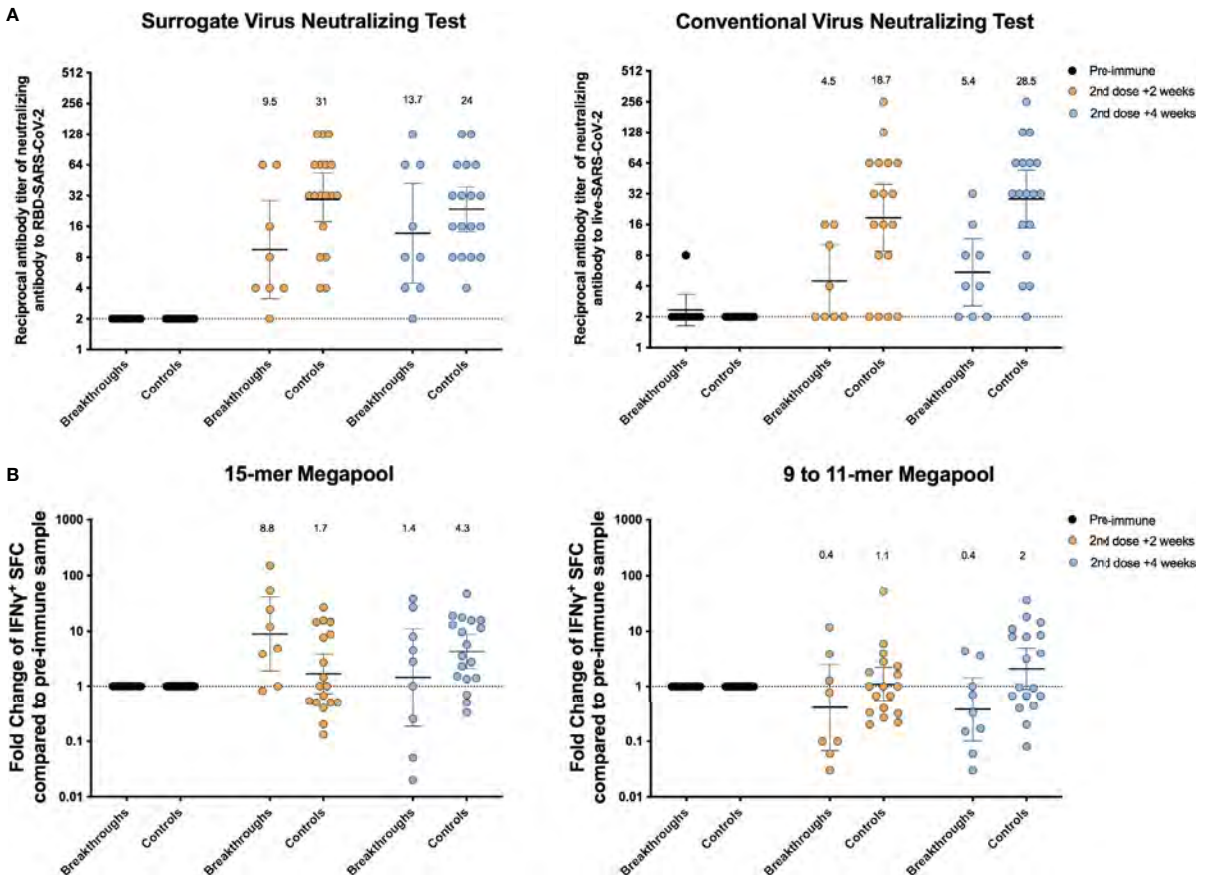


FIGURE 5 | Humoral and cellular immune responses of breakthrough cases as compared to a control cohort. A control cohort of 18 subjects who received two doses of the CoronaVac was selected by matching with breakthrough cases (2:1 ratio) according to the biological sex, range of age, and schedule of vaccination. **(A)** Titers of antibodies able to inhibit RBD-SARS-CoV-2 interaction with ACE2 receptor or surrogate virus neutralizing test (sVNT, left) and titers of neutralizing antibodies against infective SARS-CoV-2 or conventional virus neutralizing test (cVNT, right) detected in the breakthrough and control cohort. Serum samples were obtained before administration of the first dose (preimmune), 2 and 4 weeks after the second dose. The numbers above the spots indicate GMT, and error bars show the 95% CI of the GMT. **(B)** Fold change of IFN-γ^+ SFCs after stimulation of PBMCs with MPs containing 15-mer peptides (left) and 9- to 11-mer MPs (right) from SARS-CoV-2 proteome in the breakthrough and control cohort. PBMCs were obtained before administration of the first dose (preimmune), 2 and 4 weeks after the second dose. The numbers above the spots indicate geometric mean of the fold increase regarding to the preimmune sample, and error bars show the 95% CI. GMT, geometric mean titer; PBMCs, peripheral blood mononuclear cells; MPs, megapools.

prone to severe cases of COVID-19 and deaths than women, and this is even more pronounced in older populations (26, 27). Overweight and obesity are one of the most common comorbidities reported in critical patients suffering severe cases of COVID-19 (28). Furthermore, it has been reported that patients with elevated BMI exhibit more severe infection than patients with normal BMI (a high BMI is usually defined as ≥25) (29). This point is critical, as Volunteers 4 and 7 had a BMI of 28·0 and 29·3, respectively.

The particular bad evolution presented by Volunteer 7 could be partially explained by his underlying hypertension, and its corresponding treatment, which could induce an overexpression of angiotensin-converting enzyme 2 (ACE2), the receptor used by SARS-CoV-2 to infect target cells (30). Cardiac diseases have also been strongly associated with an increase in the susceptibility of SARS-CoV2 infection, the severity of COVID-19, and the susceptibility to death, as drugs used to control these illness may result in the overexpression of ACE2 in the heart (31, 32).

The hypothyroidism reported for Volunteer 4 has been related to increased susceptibility to severe COVID-19, as it affects the expression of ACE2 (33). Hypothyroidism may also be a factor predisposing the development of cardiac diseases, which increase the susceptibility of SARS-CoV-2 infection (33). As Volunteer 4 reported fewer comorbidities than Volunteer 7 (and therefore probably less risk factors to acquire SARS-CoV-2 and develop more severe COVID-19), a better prognosis would have been expected, which is in line with the information reported here.

Two volunteers out of the nine breakthrough cases did not exhibit a detectable immune response after immunization with CoronaVac. Volunteers 2 and 6 were younger than 60 years old

and were of different sex. Volunteer 2 was a male with overweight (BMI, 28.9) and several comorbidities such as hypothyroidism arterial hypertension, coronary heart disease, fatty liver disease and dyslipidemia. He also reported a medical history of several infectious diseases in his childhood and adulthood. The circulating antibodies of this volunteer showed a poor neutralizing capacity, and there was a practically null induction of IFN-γ-secreting T cells after both vaccination doses and even after infection with SARS-CoV-2. Despite this, the degree of the disease reported in this subject was mild, and he did not require hospitalization or oxygen assistance, but it is possible that innate immunity also played a key role in the protection of this individual or that antigen-specific adaptative immune responses were not detected, since they could be restricted to mucosae or lungs (34, 35). Volunteer 6 was a female with normal weight and comorbidities such as hypothyroidism. The circulating antibodies of this volunteer showed a poor neutralizing capacity, but unlike Volunteer 2, she developed a robust cellular response after 4 weeks of vaccination which was also increased after disease onset. Although the number of breakthrough cases between both immunization schedules are not balanced, it is important to note that Volunteer 2 and 6 were vaccinated in the 0–14 schedule, which has been reported to induce a lower seroconversion rate and GMTs than the 0–28 schedule (36). Interestingly, both volunteers had hypothyroidism as a common comorbidity, which could affect the induction of the immune response and produce a dysregulation of the immune system (37). In this line, more in-depth studies are required to understand which factors could be involved in these poor responses and how they could impact in the future with the appearance of new circulating variants of SARS-CoV-2.

Limitations of this study include the sample size and the focus on self-reporting to identify breakthrough vaccine infections. Asymptomatic infections were not discarded and could therefore be missed in the cohort chosen as control, which in turn may cause a misinterpretation of the results regarding the comparison with the immune response elicited by the breakthrough cases. Therefore, our conclusions are directed toward the correlation of protection to suffer a symptomatic infection. On the other hand, only in Volunteer 4 the Gamma variant was identified by molecular analysis, and these data remained unknown for the rest of the breakthrough cases analyzed (Volunteer 6, 7, and 9). Hence, we lack evidence to determine whether the frequency of breakthrough vaccine cases is related to community transmission of a particular variant, which, in the case of Chile, has been dominated by the SARS-CoV-2 variants Gamma and Lambda in recent months (38).

Despite the low number of breakthrough cases included in this report, our results provide a clear and extensive clinical and immune description of mild, moderate, or severe infections exhibited after full vaccination with CoronaVac and support previous evidence that a poor induction of neutralizing antibodies after vaccination could be correlated to a decrease in the vaccine efficacy (39–41). Furthermore, data presented here provide valuable information over the potential role that play the underlying comorbidities on the vaccine effectiveness, which could impair the ability of an individual to activate a robust immune response after vaccination, and increase the risk of severe COVID-19 in elderly people. This information could be helpful and timely support the need of a booster dose in susceptible individuals with underlying conditions after a specific time to increase its protection.

Although the information presented here must be interpreted with caution because the sample size is small to generalize, some strengths of our study are worth noting, such as the serial testing after vaccination and infection and the measurement of T-cell responses in addition to humoral response. Previous reports have been focused on viral sequence information or antibodies detection on samples obtained after the onset of symptoms (11, 12, 39, 42, 43). This new information could be the interest to the scientific community and health authorities due to the urgent need to understand the individual variables that predispose to breakthrough infections and further find a correlate of protection that has not been established to date for SARS-CoV-2 infections; yet, some studies suggest that the level of neutralizing antibody titers is highly predictive of immune protection (40, 41). In this regard, our serial sample data reveal some key features: first, older volunteers 4 and 7 who presented moderate and severe illness, respectively, displayed the weakest humoral response after vaccination, but conversely, they showed the highest level of neutralizing antibodies titers after infection. Notably, susceptibility to infection was irrespective of the immunization schedule, as one of them belonged to the 0–14 immunization schedule and the other one to the 0–28. Second, younger people could not be able to elicit a good humoral immune response after vaccination or subsequent infection, as shown by volunteers 2 and 6. These observations could be explained, at least in part, by the presence of some comorbidities in these individuals and highlighted the importance of combining clinical information along with immunogenicity and efficacy studies. Finally, individuals with evidence of neutralizing antibodies elicited by vaccination can also become sick, but this is more likely to course with a mild infection (Volunteers 1, 3, 5, 8, and 9). Importantly, we observed that the level of neutralizing antibodies in this breakthrough cohort was lower than that in controls without a confirmed SARS-CoV-2 infection, but it remains to be determined what titers of antibodies are needed to prevent infection.

On the other hand, since the approval for the emergency use of CoronoVac, the WHO has encouraged addressing the current knowledge gap about the vaccine efficacy through assessment and reporting of breakthrough infections by using neutralization and T-cell immunity assays (44). To our knowledge, this is the first time that cellular-mediated response is reported for breakthrough vaccine cases. Our results showed that breakthrough cases had a good T-cell response elicited after vaccination but that was more associated to $CD4^+$ than $CD8^+$ T cells. A similar response was observed after infection, with only a volunteer not responding (Volunteer 2). It is important to note that not only cellular response to spike protein was evidenced but also to others viral antigens, as shown after stimulation with the megapool R (**Supplementary Figure 3**). However, it is not clear whether both humoral and T-cells responses are needed for protection, and further studies are needed to address that issue.

In summary, vaccination with CoronaVac is effective, and vaccine breakthrough cases showed mainly mild symptoms of COVID-19, even in those who did not exhibit a potent humoral immune response, which could be possibly associated with different risk factors as overweight and other comorbidities that could impair the immune response induced upon immunization. While additional data have become available to draw more robust conclusions, this evidence and information could be useful to the countries that actually have implemented CoronaVac in their vaccination campaigns and to guide future vaccination program policies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité Ético Científico de Ciencias de la Salud UC, Pontificia Universidad Católica de Chile. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: AK, KA, SB, PG, JG-A, GZ, and WM. Visualization: AK, KA, SB, PG, JG-A, GZ, and WM. Methodology: RF and JM. Investigation: LD, NG, CI, FM-G, JS, BS, MU, RB-R, LG, GH-E, DM-T, GAP, MR, DR-P, OV, YV, MN, and ÁR. Funding acquisition: AK. Project administration: AK, KA, SB, and PG. Supervision: AK, KA, SB, and PG. Writing—original draft: LD, NG, JS, CI, and MU. Writing—review and editing: AK, KA, SB, and PG. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by The Ministry of Health, Government of Chile and the Confederation of Production and Commerce (CPC), Chile, through the funding of the CoronaVac03CL Study. The Millennium Institute on Immunology and Immunotherapy, ANID—Millennium Science Initiative Program ICN09_016 (former P09/016-F), supports SB, KA, PG, and AK. The Innovation Fund for Competitiveness FIC-R 2017 (BIP Code: 30488811-0) supports SB, PG, and AK. SINOVAC contributed to this study with the investigational vaccine and experimental reagents.

ACKNOWLEDGMENTS

We would like to thank the support of the Ministry of Health, Government of Chile; Ministry of Science, Technology, Knowledge, and Innovation, Government of Chile; and the Ministry of Foreign Affairs, Government of Chile and the Chilean Public Health Institute (ISP). We also would like to thank Rami Scharf, Jessica White, Jorge Flores, and Miren Iturriza-Gomara from PATH for their support in the experimental design and discussion. We also thank Daniela Weiskopf and Alejandro Sette for sharing the SARS-CoV-2 Megapools used in the ELISPOT assays and their assistance in T-cells stimulation experiments. We also thank Ana María Guzmán, Marcela Ferrés and Constanza Martínez from Escuela de Medicina, Pontificia Universidad Católica de Chile, for supporting in laboratory assays. We also thank the Vice Presidency of Research (VRI), the Direction of Technology Transfer and Development (DTD), the Legal Affairs Department (DAJ) of the Pontificia Universidad Católica de Chile. We are also grateful to the Administrative Directions of the School of Biological Sciences and the School of Medicine of the Pontificia Universidad Católica de Chile for their administrative support. Special thanks to the members of the CoronaVac03CL Study Team (list in the **Supplementary Appendix (SA)** for their hard work and dedication to the clinical study; the members of the Independent Data Safety Monitoring Committee (members in the SA) for their oversight, and the subjects enrolled in the study for their participation and commitment to this trial.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.742914/full#supplementary-material

Supplementary Figure 1 | Evaluation of anti-S1-RBD SARS-CoV-2 Ig-G antibodies through ELISA assays. Results are reported as the optical density value (OD_{450nm}) reached after two-fold serial dilutions, starting at 1:200. Samples were obtained before administration of the first dose (pre-immune), two and four weeks after the second dose, and two and four weeks after the disease onset (follow up 1 and 2, respectively). Dotted line indicates the cut-off for the serum dilution at 1:200. **(A–F)** Volunteers 1 to 6 belonging to the 0-14 immunization schedule. **(G–I)** Volunteers 7 to 9 belonging to the 0-28 immunization schedule.

Supplementary Figure 2 | Percentage of inhibition of hACE2-spike protein-protein interaction evaluated by a surrogate virus neutralization test (sVNT). Serum samples from nine volunteers were two-fold serially diluted starting to 1:2 and up to 4,096 for neutralizing antibodies detection. Samples were obtained before administration of the first dose (pre-immune), two and four weeks after the second dose, and two and four weeks after the disease onset (follow up 1 and 2, respectively). The dotted line represents the cut-off value at 30% of inhibition **(A–F)** Volunteers 1 to 6 belonging to the 0-14 immunization schedule. **(G–I)** Volunteers 7 to 9 belonging to the 0-28 immunization schedule.

Supplementary Figure 3 | T cells responses of breakthrough cases after stimulation with MPs composed of peptides from SARS-CoV-2 proteome. IFN-γ^+

SFCs of nine breakthrough cases. Data are shown as the fold increase regarding to the pre-immune value for SFCs **(A)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 15-mer peptides from the S protein of SARS-CoV-2. **(B)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 15-mer peptides from the proteome of SARS-CoV-2 excluding the S protein. **(C, D)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 9 to 11-mer peptides from the SARS-CoV-2 proteome.

Supplementary Figure 4 | Neutralizing antibody titers of 15 breakthrough cases as compared to 18 vaccinated subjects with no evidence of symptoms associated with COVID-19. Serum samples of individuals were evaluated before vaccine administration (pre-immune), two and four weeks after the second dose. Neutralizing antibodies titers were determined by using **(A)** a surrogate virus neutralizing test and **(B)** a conventional virus neutralizing test. The numbers above the spots indicate the geometric mean titer (GMT) and error bars show the 95% CI of the GMT.

REFERENCES

1. Dong E, Du H, Gardner L. An Interactive Web-Based Dashboard to Track COVID-19 in Real Time. *Lancet Infect Dis* (2020) 20:533–4. doi: 10.1016/S1473-3099(20)30120-1
2. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus From Patients With Pneumonia in China, 2019. *N Engl J Med* (2020) 382:727–33. doi: 10.1056/NEJMoa2001017
3. Dixon BE, Wools-Kaloustian KK, Fadel WF, Duszynski TJ, Yiannoutsos C, Halverson PK, et al. Symptoms and Symptom Clusters Associated With SARS-CoV-2 Infection in Communitybased Populations: Results From a Statewide Epidemiological Study. *PloS One* (2021) 16:1–13 . doi: 10.1371/journal.pone.0241875
4. Krammer F. SARS-CoV-2 Vaccines in Development. *Nature* (2020) 586:516–27. doi: 10.1038/s41586-020-2798-3
5. WHO. *Covid-19. Draft Landscape of COVID-19 Candidate Vaccines*. WHO (2020).
6. Kyriakidis NC, López-Cortés A, González EV, Grimaldos AB, Prado EO. SARS-CoV-2 Vaccines Strategies: A Comprehensive Review of Phase 3 Candidates. *NPJ Vaccines* (2021) 6:1–17. doi: 10.1038/s41541-021-00292-w
7. Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, Tolerability, and Immunogenicity of an Inactivated SARS-CoV-2 Vaccine in Healthy Adults Aged 18-59 Years: A Randomised, Double-Blind, Placebo-Controlled, Phase 1/2 Clinical Trial. *Lancet Infect Dis* (2021) 21:181–92. doi: 10.1016/S1473-3099(20)30843-4
8. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and Immunogenicity of the ChAdOx1 Ncov-19 Vaccine Against SARS-CoV-2: A Preliminary Report of a Phase 1/2, Single-Blind, Randomised Controlled Trial. *Lancet* (2020) 396:467–78. doi: 10.1016/S0140-6736(20)31604-4
9. Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Phase I/II Study of COVID-19 RNA Vaccine BNT162b1 in Adults. *Nature* (2020) 586:589–93. doi: 10.1038/s41586-020-2639-4
10. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* (2020) 383:2603–15. doi: 10.1056/nejmoa2034577
11. Philomina JB, Jolly B, John N, Bhoyar RC, Majeed N, Senthivel V, et al. Genomic Survey of SARS-CoV-2 Vaccine Breakthrough Infections in Healthcare Workers From Kerala, India. *J Infect* (2021) 83:237–79. doi: 10.1016/j.jinf.2021.05.018
12. Hacisuleyman E, Hale C, Saito Y, Blachere NE, Bergh M, Conlon EG, et al. Vaccine Breakthrough Infections With SARS-CoV-2 Variants. *N Engl J Med* (2021) 384:2212–8. doi: 10.1056/nejmoa2105000
13. Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *N Engl J Med* (2021), 385:1–11. doi: 10.1056/NEJMoa2107715
14. Wu Z, Hu Y, Xu M, Chen Z, Yang W, Jiang Z, et al. Safety, Tolerability, and Immunogenicity of an Inactivated SARS-CoV-2 Vaccine (CoronaVac) in Healthy Adults Aged 60 Years and Older: A Randomised, Double-Blind, Placebo-Controlled, Phase 1/2 Clinical Trial. *Lancet Infect Dis* (2021) 21:803–12. doi: 10.1016/S1473-3099(20)30987-7
15. Bueno SM, Abarca K, González PA, Gálvez NM, Soto JA, Duarte LF, et al. Interim Report: Safety and Immunogenicity of an Inactivated Vaccine Against Sars-Cov-2 in Healthy Chilean Adults in A Phase 3 Clinical Trial 2 3 Brief Title: Coronavac03cl Phase 3 Interim Analysis of Safety and Immunogenicity. *medRxiv* (2021) 2021.03.31.21254494. doi: 10.1101/2021.03.31.21254494
16. Palacios R, Batista AP, Albuquerque CSN, Patiño EG, Santos J do P, Tilli Reis Pessoa Conde M, et al. Efficacy and Safety of a COVID-19 Inactivated Vaccine in Healthcare Professionals in Brazil: The PROFISCOV Study. *SSRN Electron J* (2021). doi: 10.2139/ssrn.3822780
17. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans With COVID-19 Disease and Unexposed Individuals. *Cell* (2020) 181:1489–501.e15. doi: 10.1016/j.cell.2020.05.015
18. Valdespino Gómez JL, García García MDL. Declaración De Helsinki. *Gac Med Mex* (2001) 137:387–90.
19. WHO. Obesity and Overweight. In: *World Heal Organ Media Cent Fact Sheet No 311*. WHO (2021).
20. U. S. Department of Health and Human Services, Services USD of H and H, Administration F and D and Research C for BE. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. *Guid Ind* (2007).
21. National Cancer Institute. *Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0*. NIH Publ (2017). Available at: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm.
22. WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A Minimal Common Outcome Measure Set for COVID-19 Clinical Research. *Lancet Infect Dis* (2020) 20:e192–7. doi: 10.1016/S1473-3099(20)30483-7
23. Kluge DHHP. *Statement – Older People are at Highest Risk From COVID-19, But All Must Act to Prevent Community Spread*. World Heal Organ (2020). Available at: https://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/statements/statement-older-people-are-at-highest-risk-from-covid-19,-but-all-must-act-to-prevent-community-spread.
24. Kwetkat A, Heppner HJ. Comorbidities in the Elderly and Their Possible Influence on Vaccine Response. *Interdiscip Top Gerontol Geriatr* (2020) 43:73–85. doi: 10.1159/000504491
25. Liu K, Chen Y, Lin R, Han K. Clinical Features of COVID-19 in Elderly Patients: A Comparison With Young and Middle-Aged Patients. *J Infect* (2020) 80:e14–8. doi: 10.1016/j.jinf.2020.03.005
26. Jin JM, Bai P, He W, Wu F, Liu XF, Han DM, et al. Gender Differences in Patients With COVID-19: Focus on Severity and Mortality. *Front Public Heal* (2020) 8:1–6. doi: 10.3389/fpubh.2020.00152
27. Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering How Biological Sex Impacts Immune Responses and COVID-19 Outcomes. *Nat Rev Immunol* (2020) 20:442–7. doi: 10.1038/s41577-020-0348-8
28. Kassir R. Risk of COVID-19 for Patients With Obesity. *Obes Rev* (2020) 21:1–2. doi: 10.1111/obr.13034
29. Liu M, He P, Liu HG, Wang XJ, Li FJ, Chen S, et al. Clinical Characteristics of 30 Medical Workers Infected With New Coronavirus Pneumonia. *Zhonghua Jie He He Hu Xi Za Zhi* (2020) 43:209–14. doi: 10.3760/cma.j.issn.1001-0939.2020.03.014
30. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, et al. Effect of Angiotensin-Converting Enzyme Inhibition and Angiotensin II Receptor Blockers on Cardiac Angiotensin-Converting Enzyme 2. *Circulation* (2005) 111:2605–10. doi: 10.1161/CIRCULATIONAHA.104.510461
31. Kow CS, Zaidi STR, Hasan SS. Cardiovascular Disease and Use of Renin-Angiotensin System Inhibitors in COVID-19. *Am J Cardiovasc Drugs* (2020) 20:217–21. doi: 10.1007/s40256-020-00406-0
32. Zhang Y, Gao Y, Qiao L, Wang W, Chen D. Inflammatory Response Cells During Acute Respiratory Distress Syndrome in Patients With Coronavirus Disease 2019 (COVID-19). *Ann Intern Med* (2020) 173:402–4. doi: 10.7326/L20-0227
33. Brix TH, Hegedüs L, Hallas J, Lund LC. Risk and Course of SARS-CoV-2 Infection in Patients Treated for Hypothyroidism and Hyperthyroidism. *Lancet Diabetes Endocrinol* (2021) 9:197–9. doi: 10.1016/S2213-8587(21)00028-0
34. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-Cell Landscape of Bronchoalveolar Immune Cells in Patients With COVID-19. *Nat Med* (2020) 26:842–4. doi: 10.1038/s41591-020-0901-9
35. Su Y, Chen D, Yuan D, Lausted C, Choi J, Dai CL, et al. Multi-Omics Resolves a Sharp Disease-State Shift Between Mild and Moderate COVID-19. *Cell* (2020) 183:1479–95.e20. doi: 10.1016/j.cell.2020.10.037

36. Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, Tolerability, and Immunogenicity of an Inactivated SARS-CoV-2 Vaccine in Healthy Adults Aged 18–59 Years: A Randomised, Double-Blind, Placebo-Controlled, Phase 1/2 Clinical Trial. *Lancet Infect Dis* (2021) 21:181–92. doi: 10.1016/S1473-3099(20)30843-4
37. Hariyanto TI, Kurniawan A. Thyroid Disease Is Associated With Severe Coronavirus Disease 2019 (COVID-19) Infection. *Diabetes Metab Syndr Clin Res Rev* (2020) 14:1429–30. doi: 10.1016/j.dsx.2020.07.044
38. Acevedo ML, Alonso-Palomares L, Bustamante A, Gaggero A, Paredes F, Cortés CP, et al. Infectivity and Immune Escape of the New SARS-CoV-2 Variant of Interest Lambda. *medRxiv* (2021) 2021.06.28.21259673. doi: 10.1101/2021.06.28.21259673
39. Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, et al. Covid-19 Breakthrough Infections in Vaccinated Health Care Workers. *N Engl J Med* (2021), 1–11. doi: 10.1056/nejmoa2109072
40. Earle KA, Ambrosino DM, Fiore-Gartland A, Goldblatt D, Gilbert PB, Siber GR, et al. Evidence for Antibody as a Protective Correlate for COVID-19 Vaccines. *Vaccine* (2021) 39:4423–8. doi: 10.1016/j.vaccine.2021.05.063
41. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing Antibody Levels Are Highly Predictive of Immune Protection From Symptomatic SARS-CoV-2 Infection. *Nat Med* (2021) 27:1205–11. doi: 10.1038/s41591-021-01377-8
42. Estofolete CF, Banho CA, Campos GRF, Marques BC, Sacchetto L, Ullmann LS, et al. Case Study of Two Post Vaccination SARS-CoV-2 Infections With P1 Variants in Coronavac Vaccinees in Brazil. *Viruses* (2021) 13:1–10. doi: 10.3390/v13071237
43. Nixon DF, Ndhlovu LC. Vaccine Breakthrough Infections With SARS-CoV-2 Variants. *N Engl J Med* (2021) 385:e7. doi: 10.1056/nejmc2107808
44. Strategic Advisory Group of Experts on Immunization - SAGE (WHO). *Interim Recommendations for Use of the Inactivated COVID-19 Vaccine, CoronaVac, Developed by Sinovac* (2021). Available at: https://apps.who.int/iris/bitstream/handle/10665/341454/WHO-2019-nCoV-vaccines-SAGE-recommendation-Sinovac-CoronaVac-2021.1-eng.pdf.

Conflict of Interest: ZG and MW are SINOVAC employees and contributed to the conceptualization of the study (clinical protocol and eCRF design) and did not participate in the analysis or interpretation of the data presented in the manuscript.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.7. Estudo sugere que populações de países da América do Sul que usaram CoronaVac estão protegidas contra variantes gama e lambda

Um estudo de pesquisadores do Brasil e do Uruguai sugere que as populações dos países do sul da América do Sul estão mais protegidas contra as variantes regionais gama e lambda do vírus SARS-CoV-2. As conclusões estão em um artigo científico publicado na plataforma de preprints MedRxiv. Segundo os pesquisadores, da Universidade da República, de Montevidéu, e da Fundação Oswaldo Cruz (Fiocruz), do Rio de Janeiro, a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, na condição de vacina inativada, contribuiu decisivamente para esse resultado.

De acordo com o estudo, Argentina, Brasil, Chile, Paraguai e Uruguai experimentaram ondas epidêmicas graves de Covid-19 no início de 2021, impulsionadas pela expansão das variantes gama e lambda. No entanto, a partir de junho, houve uma melhora nos indicadores da epidemia. Na 14ª semana epidemiológica, entre 4 e 10 de abril, foram registrados 21.141 óbitos por Covid-19, segundo o Painel Coronavírus do Ministério da Saúde. Foi a maior quantidade de mortes registrada em sete dias no ano inteiro. Já na 25ª semana epidemiológica, entre 20 e 26 de junho, o número de mortes havia se reduzido para 11.935. Desde então, o indicador continua caindo e, na última semana epidemiológica, entre 19 e 25 de setembro, o número de mortes por Covid-19 no Brasil foi de 3.692.

O estudo afirma que o uso generalizado da CoronaVac no sul da América do Sul foi não só eficaz para prevenir as formas graves da Covid-19, mas também conteve a disseminação das variantes regionais altamente transmissíveis. No Chile, 70% das vacinas aplicadas correspondem à CoronaVac; no Uruguai, 60%; no Brasil, 35%. Vale lembrar que até meados de maio, a vacina do Butantan respondia por cerca de sete a cada dez imunizantes aplicados.

Para investigar os resultados dos programas nacionais de vacinação e o impacto da infecção natural na transmissão viral dos países do Cone Sul, os pesquisadores analisaram a associação entre a mobilidade da população e o número efetivo de reprodução (Rt) – número médio de pessoas infectadas em um determinado momento por um indivíduo infectado introduzido em uma população parcialmente imune ou suscetível (ou seja, no início da epidemia).

As análises revelaram que, de janeiro a maio de 2021, a mobilidade da população na Argentina, Brasil, Chile, Paraguai e Uruguai esteve relacionada ao número efetivo de reprodução Rt. A partir de junho, no entanto, a taxa de transmissão viral começou a ser menor do que o esperado conforme os níveis de interação social. "O estudo sugere que as populações do Cone Sul da América do Sul provavelmente alcançaram o HIT [limiar condicional de imunidade de rebanho, em inglês herd immunity threshold] para conter a transmissão das variantes gama e lambda do SARS-CoV-2 por volta de meados de 2021", afirmam os pesquisadores. As análises dos cientistas indicam que o limiar de imunidade HIT para o vírus da Covid-19, na América do Sul, variou entre 29% na Argentina, 33% no Uruguai, 36% no Paraguai, 43% no Chile e 45% no Brasil.

Os pesquisadores sugerem que os níveis de imunidade natural elevados identificados nos países da América do Sul podem ser uma condição importante que esteja contribuindo para limitar a expansão da variante. Segundo informações dos especialistas, a contribuição desta imunidade é resultado da infecção natural associada à vacinação.

Publicado em: 21/09/2021

Artigo

SARS-CoV-2 epidemic in the South American Southern cone: can combined immunity from vaccination and infection prevent the spread of Gamma and Lambda variants while easing restrictions?

Marcelo Fiori[*†] Gonzalo Bello[‡] Nicolás Wschebor[§] Federico Lecumberry[¶]
Andrés Ferragut[‖] Ernesto Mordecki[**]

September 15, 2021

Abstract

All South American countries from the Southern cone (Argentina, Brazil, Chile, Paraguay and Uruguay) experienced severe COVID-19 epidemic waves during early 2021 driven by the expansion of variants Gamma and Lambda, however, there was an improvement in different epidemic indicators since June 2021. To investigate the impact of national vaccination programs and natural infection on viral transmission in those South American countries, we analyzed the coupling between population mobility and the viral effective reproduction number R_t. Our analyses reveal that population mobility was highly correlated with viral R_t from January to May 2021 in all countries analyzed; but a clear decoupling occurred since May-June 2021, when the rate of viral spread started to be lower than expected from the levels of social interactions. These findings support that populations from the South American Southern cone probably achieved the conditional herd immunity threshold to contain the spread of regional SARS-CoV-2 variants.

1 Introduction

Countries from the South America Southern cone experienced large COVID-19 epidemic waves during the first months of 2021 driven by the lack of stringent mitigation measures along with the emergence and regional spread of the Variant of Concern (VOC) Gamma and the Variant of Interest (VOI) Lambda [1]. The VOC Gamma was the predominant viral variant in Brazil, Paraguay and Uruguay; while both Gamma and Lambda circulated at similar prevalence in Argentina and Chile [2, 3, 4, 5]. Changes in different epidemic indicators from mid-June to end of August, including declining numbers of new SARS-CoV-2 cases and deaths and viral effective reproduction number R_t below one, support a relative control of the COVID-19 epidemic in all five countries [1]. The drivers of such epidemic control remained unclear as SARS-CoV-2 transmission could be influenced by several factors including extent of non-pharmaceutical interventions (NPIs), level of social distancing, adherence to self-care measures, transmissibility of circulating viral variants and the proportion of susceptible host [6].

Several studies demonstrate that during the pre-vaccination phase and in a context of large community transmission of the virus, when other factors as contact tracing strategies are not effective, changes in population mobility could be predictive of changes in epidemic trends and viral R_t [7, 8, 9, 10, 11, 12, 13]. In those settings, decoupling between population mobility and viral transmissions could be used as a surrogate marker of herd immunity achieved either through high vaccination and/or natural infection rates. Data from countries with advanced vaccination like Israel and the United Kingdom support this

[*]Corresponding author: mfiori@fing.edu.uy
[†]Instituto de Matemática y Estadística "Rafael Laguardia", Facultad de Ingeniería, Universidad de la República, Uruguay
[‡]Instituto Oswaldo Cruz (FIOCRUZ), Rio de Janeiro , Brazil
[§]Instituto de Física, Facultad de Ingeniería, Universidad de la República, Uruguay
[¶]Instituto de Ingeniería Eléctrica, Facultad de Ingeniería, Universidad de la República, Uruguay
[‖]Facultad de Ingeniería, Universidad ORT, Uruguay
[**]Centro de Matemática, Facultad de Ciencias, Universidad de la República, Uruguay

notion as in a certain time SARS-CoV-2 incidence display sustained declines despite easing of lockdown restrictions, discontinuation of face mask use in open spaces and increase in population mobility [14, 15]

In the present article, we estimate the coupling between population mobility and the R_t of SARS-CoV-2 in the five South American countries from the Southern cone. Our analyses support that mobility data was highly correlated with the viral R_t in all South American countries analyzed between January and May, 2021; however, a clear decoupling between population mobility and viral transmissions was evident since May-June 2021. The mean estimated threshold of immune individuals (fully vaccinated pondered by vaccine effectiveness plus natural infected) necessary to produce such decoupling varies along the five countries from 29% to 45% and a discussion trying to understand these differences is provided. These findings also support the relevance of vaccination-induced herd immunity in South American countries with widespread use of the inactivated vaccine Coronavac.

2 Results

To analyze the potential correlation between social mobility and the spread of the SARS-CoV-2, we estimate the viral effective reproduction number R_t in every country based on mobility information provided by Google [16] during a time period of high viral transmission (see subsection 4.2). The resulting estimator, denoted as $\hat{R}_t$, was then correlated with the observed R_t estimated from the incidence data available in the Our World in Data (OWID) data base [1]. The correlation between $\hat{R}_t$ and R_t provides a measure of the value of social mobility as a predictor of viral transmissions in each country, while the ratio $\hat{R}_t/R_t$ provides a measure of the coupling between both indicators. In all five South American countries analyzed (Argentina, Brazil, Chile, Paraguay and Uruguay) we observed that during the first months of 2021, the estimated $\hat{R}_t$ was highly correlated (ρ^2 between 0.83 y 0.94) with the observed R_t about 1-2 weeks later and the ratio $\hat{R}_t/R_t$ was close to one (0.90-1.10) during the pre-vaccination and initial vaccination phases (Figure 1). We observed a high correlation between both estimators not only during the estimation period, but also during the beginning of the vaccination roll-out. These findings confirm that population mobility was a relevant driver of viral transmissions during early 2021 in all South American countries analyzed and revealed that, under a context of high community transmission, researchers can use the observed population mobility at a given time to infer the viral transmission dynamics without the typical lag of the observed R_t.

When we extended the estimation of the $\hat{R}_t$ during the vaccination roll-out period (with the same computed initial parameters), we observed a clear increase of the ratio $\hat{R}_t/R_t$ in all South American countries analyzed since late May and early June 2021, indicating that at a certain time the rate of spread of the virus started to be lower than expected from the levels of social interactions (Figure 1). We interpret such decoupling between population mobility and viral spread as a surrogate marker of conditional herd immunity, i.e. the achieved herd immunity conditioned to the social distancing policies and the circulating viral variants in each country. In order to test our method, we conducted a similar analysis in Israel, the first country to attain conditional vaccine-induced herd immunity. Our findings confirm that after a period of clear coupling between population mobility and viral transmission, a decisive increase of the ratio $\hat{R}_t/R_t$ was also observed at a certain time during vaccination roll-out in Israel (Figure A.1). The decoupling time, defined as the moment when the ratio $\hat{R}_t/R_t$ finally overcomes (i.e. the last time it crosses) the value 1.10, preceded the last peak of weekly reported cases and roughly coincides with the last day when the $R_t = 1$ in each country (Figure 1), indicating that the decoupling time was an early indicator of epidemic control.

The proportion of immunized population at the decoupling time could give us an idea of the conditional herd immunity threshold (HIT). In order to estimate the proportion of immune individuals around the decoupling time, we summed the estimated number of vaccine-immunized and natural-immunized individuals. The proportion of vaccine-immunized individuals was estimated from the number of fully vaccinated individuals adjusted by the estimated vaccine effectiveness (VE) in South America [17, 18], see also [19]. The number of infected people that acquired immunity through previous infection (cumulative infection) was estimated from the cumulative number of deaths assuming a constant (age adjusted) infection fatality rate (IFR) for each country (see subsection 4.1 and Table 1). The mean estimated HIT at the decoupling time varies along the countries from 29% in Argentina to 33% in Uruguay, 36% in Paraguay, 43% in Chile and 45% in Brazil, although confidence

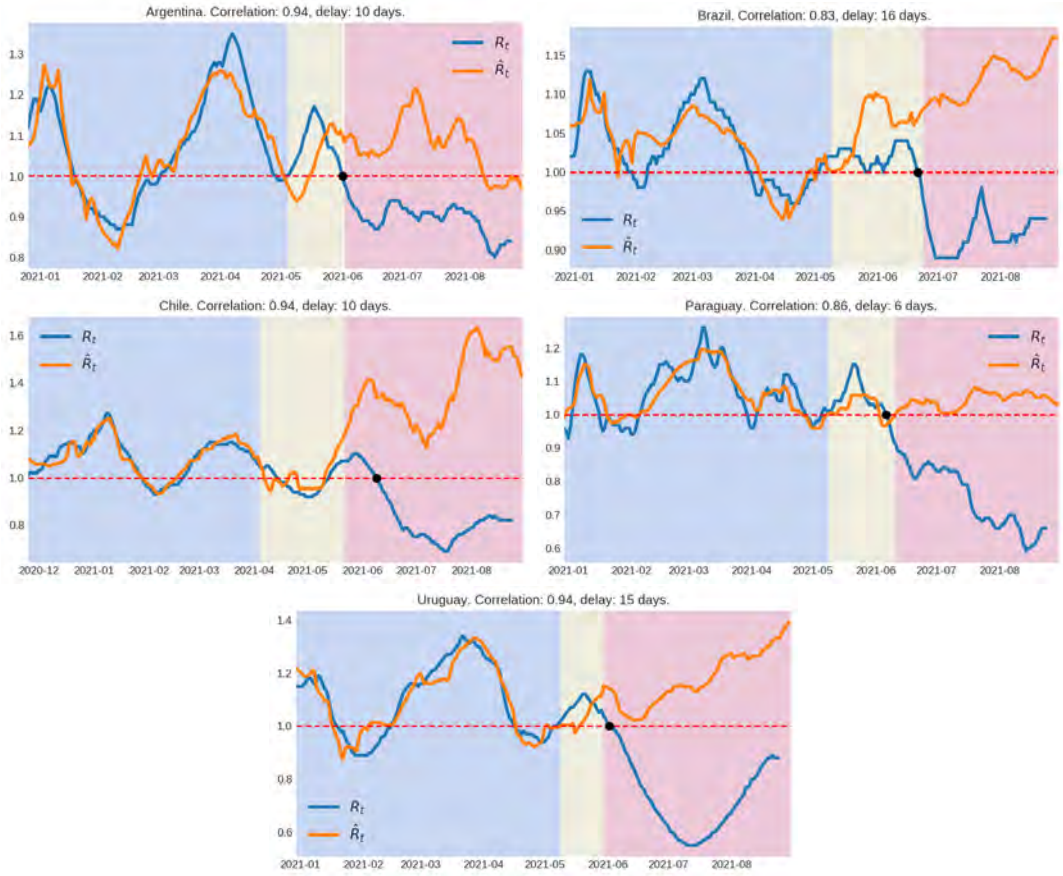


Figure 1: Viral effective reproduction number R_t and its estimation $\hat{R}_t$ using mobility information. Background colors indicate the following time periods: in blue, the time period used to fit the linear model (see Section 4.2), in yellow, the period after the fitting, but before the decoupling time, and in red after the decoupling point. The black dot corresponds to the last time the reproductive number was above one. The correlation corresponds to the period used to fit the model. The delay indicated is the time-shift between the mobility time series and R_t in order to maximize the correlation in the linear regression.

intervals were very large due to uncertainties in the IFR estimates (Table 1 and Figure 2). The HIT was reached by different proportions of natural infections and vaccination (Table 1). The estimated proportion of individuals that acquired immunity through vaccination (taking into account the VE) at the decoupling time was relatively high in Chile (29%) and Uruguay (24%), but very low in Brazil (9%), Argentina (5%) and Paraguay (1%). The estimated HIT in countries with widespread use of the inactivated vaccine Coronavac like Chile (43%) and Uruguay (33%) was similar to that estimated in Israel (42%) that only used the BNT162b2 (mRNA-based) vaccine (Figure A.2).

3 Discussion

All countries from the South America Southern cone (Argentina, Brazil, Chile, Paraguay and Uruguay) witnessed pronounced increases in daily SARS-CoV-2 cases and deaths during the firsts months of 2021 and a clear drop in relevant epidemic metrics (cases, deaths and R_t) from mid-2021 [1]. This study demonstrates that such epidemic control was preceded by a clear decoupling of viral transmissions from population mobility, consistent with the notion that those South American countries probably attained the HIT against SARS-CoV-2 variants Gamma and Lambda prevalent in the region, given some level of social dis-

Country	IFR	(VIN, ADV, RNA)	Dec-T	% Nat-Inf	% Vac	HIT
Argentina	0.67 (0.36-1.30)	(31.1, 64.7, 04.2)	Jun. 02	26 (13-48)	06	29 (17-52)
Brazil	0.59 (0.32-1.17)	(34.4, 48.1, 17.5)	Jun. 23	40 (20-74)	11	45 (25-79)
Chile	0.73 (0.40-1.43)	(71.1, 06.9, 22.0)	May 22	20 (10-37)	40	43 (34-60)
Paraguay	0.41 (0.23-0.83)	(11.6, 26.6, 61.8)	Jun. 11	35 (18-64)	02	36 (19-64)
Uruguay	0.90 (0.49-1.56)	(59.8, 01.6, 38.6)	May 29	13 (8-24)	29	33 (27-44)
Israel	0.65 (0.35-1.27)	(0,0,100)	Feb. 28	10 (5-19)	39	42 (37-51)

Table 1: IFR: infection fatality rate; VIN: percentage of virus inactivated vaccines; ADV: percentage of adenovirus vaccines; RNA: percentage of RNA vaccines [20, 21, 22, 23, 24, 25]; Dec-T: decoupling time; % Nat-Inf: percentage of population naturally infected at Dec-T; % Vac: percentage of the population fully vaccinated at Dec-T; HIT (herd immunity threshold): percentage of immunized population due to vaccines and natural infections at Dec-T. The vaccine effectiveness (VE) against SARS-CoV-2 infections was adjusted to 66% for VIN, 73% for ADV and 93% for RNA [17, 18].

tancing restrictions.

At the start of the pandemic, thresholds of 60-70% were given as estimates of herd immunity for SARS-CoV-2 [26]. Despite confidence intervals of HIT estimates were very large, mostly due to uncertainties in the IFR estimates, our analyses support that the conditional HIT for SARS-CoV in South America would be lower than 50%, ranging from 29% in Argentina to 45% in Brazil. Moreover, observe that these confidence intervals have a common range of $(34, 44) = 39 \pm 5$. A recent modeling study conducted in Stockholm, Sweden, also supports that this country reached the HIT against the original and Alpha variants of SARS-CoV-2 at 23% and 33% of seroprevalence, respectively [27]. The authors conclude that HIT for SARS-CoV-2, given limited social distancing restrictions, could be lower than initially estimated and that phenomena could be explained by population heterogeneity. By fitting epidemiological models that allow for heterogeneity in susceptibility or exposure to SARS-CoV-2 and given a basic reproduction number R_0 between 2.5 and 3, a recent study estimates that the HIT declines from over 60% to less than 10% as the coefficient of variation increases [28]. Another study estimate that in an age-structured community with mixing rates fitted to social activity, the HIT can be 43% if R_0 is 2.5 [29].

Our findings also support that the conditional HIT for SARS-CoV-2 in South America was attained through both natural and vaccinal immunity, with different relative proportions across countries. The extremely low proportion of vaccine-immune individuals in Paraguay (1%), Argentina (5%) and Brazil (9%) at decoupling time, suggest that conditional herd immunity in those countries was mostly attained by natural infections. Few studies estimated the proportion of infected individuals in South America after the large Gamma and Lambda epidemics in 2021, but some evidence from seroprevalence data support our estimations. A randomized study conducted in Paraguay between March to June 2021 gave a seroprevalence of 23.1% in Asunción and of 26.9% in the central region of the country [30] and a recent seroprevalence survey among adult individuals living in the largest Brazilian city of Sao Paulo also estimate a high proportion (45%: 39-51%) of individuals infected by SARS-CoV-2 [31].

At the other extreme, the relative proportion of vaccinal immunity at decoupling was highest in Chile (29%) and Uruguay (24%). CoronaVac accounted for most of vaccinations in Chile (75%) [32] and Uruguay (66%) [24] and the high incidence of SARS-CoV-2 in those countries during first months of vaccination roll-out raise concerns about the effectiveness of inactivated virus vaccines to control SARS-CoV-2 transmissions. Our results support that the widespread use of inactivated virus vaccines contributed to containing the spread of SARS-CoV-2 in Chile and Uruguay, despite abundant circulation of VOCs/VOIs and weak mitigation measures. Remarkably, the HIT at decoupling point in Chile (43%) and Uruguay (33%) was similar to the one estimated for Israel (42%), that mostly controlled the virus expansion through vaccination with BNT162b2. These findings are consistent with recent studies of vaccine effectiveness (VE) in Chile [17], Brazil [18] and Bahrain [33] that conclude that immunization with inactivated vaccines (CoronaVac and Sinopharm) was an effective strategy at mitigating the risk for transmissions of SARS-CoV-2 VOCs, although the perfor-

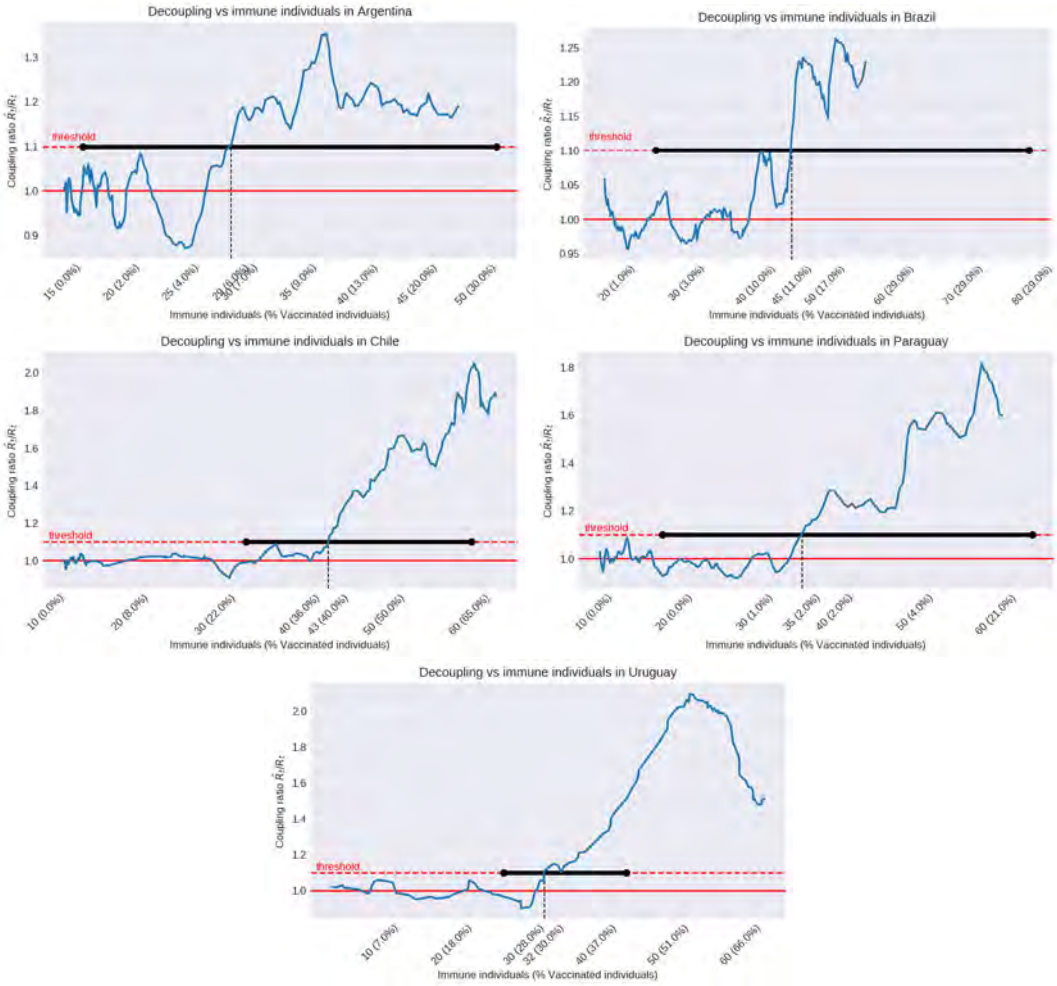


Figure 2: Coupling ratio $\hat{R}_t/R_t$ plotted with respect to the percentage of immune population. During the first months of 2021 the coupling ratio varies around 1, which corresponds to the periods where the R_t and $\hat{R}_t$ are in concordance in Figure 1. Immune population includes immunity achieved by vaccination (taking into account its effectiveness), and natural infection (see subsection 4.3). The percentage of people fully vaccinated is described as well. The coupling ratio crosses the threshold (decoupling point) at percentages of immune population that varies along the five countries from 29% in Argentina to 33% in Uruguay, 37% in Paraguay, 43% in Chile and 45% in Brazil. Confidence intervals are shown in horizontal black lines. They inherit the large uncertainty in the IFR estimation (see Table 1).

mance of BNT162b2 and adenovirus-based vaccines was superior.

The mean estimated HIT varied across South American countries and several factors may explain such variability. HIT will move upwards when more transmissible SARS-CoV-2 variants circulates in a population, but differences in the circulating SARS-CoV-2 variants do not explain variations among South American countries. Differences in the mean HIT were observed between countries where Gamma was the most prevalent variant like Brazil (45%), Paraguay (36%) and Uruguay (33%), and also between countries where Gamma and Lambda co-circulated at high prevalence like Chile (43%) and Argentina (29%) [2, 3, 4, 5]. Differences in vaccine platforms deployed in each country might also mod-

ulate the HIT at the decoupling time. Although we corrected the proportion of immune individuals according to the estimated VE and the proportion of each vaccine, we only considered immunity associated with fully vaccinated individuals. Previous studies, however, demonstrate some level of reduction of SARS-CoV-2 transmission after one dose of mRNA-based (46-58%), adenovirus-based (35%) and inactivated virus (16%) vaccines [17, 18, 34, 35]. Thus, we should expect that countries that used a higher proportion of mRNA-based and/or adenovirus-based vaccines like Argentina (69%) reached herd immunity at apparent lower thresholds that those that mostly used inactivated virus vaccines. Moreover, it should be stressed that Argentina had a very large proportion of individuals with a single dose at the decoupling point when compared to other countries in the region where second doses were administrated in a shorter period after first dose [1]. Notably, although Brazil also used an overall high proportion of mRNA-based and/or adenovirus-based vaccines (66%), most vaccinations during first months were of inactivated vaccines [18].

Reduction of SARS-CoV-2 transmission will also depend on the vaccination strategy (who is vaccinated and when). Vaccinations programs usually begin by elderly people and go on by gradually protecting the younger population [36]. Simulation studies indicate that prioritize vaccinating of high-risk groups will minimize the number of COVID-19-related hospitalizations and deaths in the short term, but vaccination of main transmission drivers (i.e. highly mobile working age groups) would be more effective at reducing the spread of the SARS-CoV-2 [37, 38]. Given enough vaccine supplies, vaccinating the adult population uniformly at random would thus be ideal to both prevent death and severe illness in high risk groups and to curb SARS-CoV-2 transmissions in the whole population. Uruguay developed an interesting vaccination strategy that prioritized vaccination of elderly populations ($\geq$ 70 years of age) with the BNT162b2 vaccine while highly mobile working age groups were simultaneously vaccinated with CoronaVac. This more homogeneous vaccination strategy across different age groups in Uruguay might partially explain the relative low HIT observed in this country. This may be related to the fact that, the decoupling effect due to vaccinations programs that we observe between mobility and the reproductive number is reached more abruptly than what could be expected from SIR-like models where all the population is treated homogeneously.

Our results support that proportion of immune population in South American populations attained a threshold enough to decoupling people mobility and viral dissemination and those countries could thus implement progressive relaxing of mitigation measures with relative safety. Such apparent herd immunity, however, was attained while maintaining moderate mitigation measures (social distancing, school closed, mask-wearing and other self-care behaviors). None of the countries analyzed have returned to the pre-pandemic levels of activity and it is unclear if current population immunity will halt the viral spread after removal of all mitigation measures. Long-term herd immunity could be also challenged by waning immunity and dissemination of more infectious SARS-CoV-2 variants [39]. Waning neutralizing antibodies might progressively reduce the population immunity level to below the critical HIT, while local evolution and/or introduction of SARS-CoV-2 variants that are more transmissible than those previous circulating will move the HIT upwards.

Both factors seems to have shaped the third epidemic wave in Israel [40, 41, 42, 43] Our study supports that after a transient period of decoupling in Israel, population mobility and viral transmissions were coupled again as Delta variant spread in both unvaccinated and fully-vaccinated individuals. It is unclear if the same phenomena could be observed in South America after introduction of Delta variant. First, herd immunity through natural infection seems to be less susceptible to waning immunity than by vaccination [44, 45, 46] and South American countries with a high natural immunity wall might be better prepared to limit the expansion of Delta variant than those with a large vaccine immunity wall. Second, hybrid immunity (natural infection plus vaccination) might provide longer lasting and stronger protection against infection than vaccine-induced immunity [47] and a high proportion of partial or fully vaccinated individuals in South America may be currently in this condition. Third, some South American countries like Chile, Uruguay and Brazil already started or approved the administration of a vaccine booster.

Our study has some important limitations: (i) difficulty to estimate precisely the IFR and consequently to have a precise estimate of the cumulative number of naturally infected people at decoupling point in each country; (ii) sub-reporting of SARS-CoV-2 deaths might underestimate the cumulative number of infections and thus the HIT; (iii) the assumption

that partially vaccinated people did not greatly contribute to reduce viral transmissions might have also underestimate the number of vaccine-immune individuals and the actual HIT; (iv) on the other hand, although we assumed some overlap between vaccinal immunity and natural immunity, the precise fraction of fully vaccinated individuals that were previously infected is unknown. Because of these limitations, the precise HIT estimated here should be interpreted with caution and should not be considered as general reference values for other countries.

In summary, our study supports that populations from the South American Southern cone probably achieved the conditional HIT to contain the further spread of SARS-CoV-2 variants Gamma and Lambda at around mid-2021. Presumed herd immunity was probably mostly attained by natural infection in Argentina, Brazil and Paraguay, and by a mixture of natural infections and vaccination in Chile and Uruguay. The widespread used of the Coronavac inactive viral vaccine in South America was not only effective to prevent the severe forms of COVID-19 disease but also has the potential to contain the community spread of highly transmissible SARS-CoV-2 regional variants. Inactivated SARS-CoV-2 vaccines, combined with other vaccines and mitigation measures, may thus represent a relevant tool to control the COVID-19 pandemic especially under the severe limitation of vaccine supplies faced by many countries around the world. Our findings stress that the herd immunity status might be rapidly lost if vaccine-induce neutralizing antibodies decrease over time and more transmissible SARS-CoV-2 variants are either introduced from abroad or evolved locally.

Acknowledgements

We are indebted to P. Bermolen, M. Cerminara, M. I. Fariello, F. Paganini, R. Radi, M. Reiris, A. Rojas de Arias and C. E. Schaerer, for useful discussion and support. Any errors are the responsibility of the authors.

4 Methods

4.1 Data and code availability

The SARS-CoV-2 incidence data, viral effective reproduction number R_t (also indicated as reproduction rate), confirmed deaths, vaccinated people, and other epidemiological indicators were retrieved from Our World in Data (OWID) [1]. Mobility index was estimated from the six indicators categories (retail and recreation, grocery and pharmacy, parks, transit stations, workplaces, and residential) provided by Google COVID-19 Community Mobility Reports [48]. For the sake of reproducible research, the code used to obtain all the results and figures is available at https://github.com/marfiori/covid19-decoupling.

4.2 Estimation of the viral effective reproduction number and decoupling time

As the correlations between the six different possible regressors are large, we construct indices that are more robust along time and different countries, to avoid overfitting. In order to do this, we choose for each country the three categories that give the best fit among all possible combinations. Although the categories may vary, the obtained fit quality is relatively robust over different time intervals. The six mobility time series were smoothed by averaging over a 14 days sliding window.

For each country, we selected a time period consisting of 75 days before the start of the vaccination campaign, and 55 days after, ending up with a 130-days period to carry out the estimation. Given a set of three mobility categories, we fitted a linear regression model to the viral effective reproduction number R_t, lagged a certain time period. This time shift was chosen as the lag that maximizes the correlation of the regression. This procedure was repeated for each combination of three categories among the six mobility measures provided by Google, and the combination achieving the best regression result was kept. It should be noted that, since the six categories are highly correlated, other combinations of three categories achieve similar fitting results, and therefore the chosen categories are not necessarily informative by themselves.

Using the coefficients obtained in this 130-days period, and rest of the mobility time series, we computed the predicted viral reproduction number $\hat{R}_t$. The procedure was tested using periods of different lengths for the estimation, and the results in the HIT are robust along the different experiments.

When population mobility and viral transmission are coupled, the coupling ratio $C_t = \hat{R}_t/R_t$ oscillates around one (0.90-1.10). Departing from a certain moment, the $\hat{R}_t$ becomes much higher than the R_t, re-

vealing the decoupling between population mobility and viral spread resulted. We defined the **decoupling time** D_t as the moment when the coupling ratio $C_t = \hat{R}_t/R_t$ definitely exceeds the value 1.10, i.e. the last crossing over 1.10.

4.3 Estimation of the IFR and immune population

As it is well known, the estimation of the infection fatality rate has been a hard task during all the pandemic. The cryptic circulation of the virus (due to asymptomatic infections) and different variants made that in fact this quantity varies along time and populations. Here we took into account the most relevant variable to compute it, that is the age structure of the population. We then took IFR by age taken from [49] and adjusted to the population pyramid of each of the considered countries [50]. Confidence intervals were calculated by considering the (very large) confidence intervals available from [49] and estimating the interval for the whole population as the weighted average of the positions for the maximum or minimum of the age-classes intervals. Only one exception was introduced: in the Uruguayan case, the confidence interval can be reduced because the IFR must be smaller than the Case Fatality Rate (CFR). Imposing this constraint the maximum possible value in the Uruguayan case is reduced (we obtained the CFR corresponding to July 31 from [1]) the other countries being unaffected. This IFR estimation was confirmed using an alternative methodology for the case of Uruguay, following [51], which led to similar results, but with slightly larger confidence intervals.

The percentage of immune population was computed considering the immunity achieved by vaccination (including its effectiveness), and natural infection. However, many people who gained immunity by natural infection, might have gotten vaccinated as well. In order to avoid the over estimation resulting from counting twice those subjects, we subtracted the intersection of these fractions, under the assumption that they are independent. Observe that this assumptions gives us a lower bound on the estimation of immune population.

For a given country, let us denote by FV the proportion of fully vaccinated people, by NI the proportion of people with immunity by natural infection, and by VE the vaccine effectiveness of the country, computed by combining the effectiveness of each vaccine type (VIN, ADV, RNA) using the proportion of vaccines used in the country (see Table 1). We assumed a perfect immunization due to natural infection. That is, we neglected in the present analysis the number of re-infections. Furthermore, let us denote by IM the estimation of the proportion of immunized population. Then, the computation described above is as follows:

$$IM = (FV - FV \cdot NI) \cdot VE + NI.$$

Here the product $FV \cdot NI$ accounts for the intersection of the populations, which is subtracted from the vaccinated population before the effectiveness factor is applied. As described through the text, the proportion of people with immunity by natural infection is inferred from the confirmed deaths, using the estimated IFR.

Observe that due to the vaccine effectiveness, the percentage of fully vaccinated people may by greater than the percentage of immunized population.

References

[1] Ritchie H. et al. Coronavirus Pandemic (COVID-19), (2020). Published online at OurWorldInData.org. Retrieved from: https://ourworldindata.org/coronavirus. Accessed 2021-08-28.

[2] Torres C. et al. Surveillance of SARS-CoV-2 variants in Argentina: detection of Alpha, Gamma, Lambda, Epsilon and Zeta in locally transmitted and imported cases. medRxiv 2021.07.19.21260779. https://doi.org/10.1101/2021.07.19.21260779

[3] Rego N. et al. Real-Time Genomic Surveillance for SARS-CoV-2 Variants of Concern, Uruguay. *Emerg. Infect. Dis.* **27**, 11 (2021).

[4] Ministério de Saúde. Fundaçao Oswaldo Cruz. Frequencia das pricipais linhagens do SARS-CoV-2 por mês de amostragem. Accessed 2021-09-02.

[5] Ministerio de Salud, Chile. Informe epidemiológico. Vigilancia genómica de Sars-cov-2 (COVID-19) Chile 08 de agosto de 2021. Accessed 2021-09-02.

[6] Boumans, M. Flattening the curve is flattening the complexity of covid-19. *HPLS* **43**, 18 (2021).

[7] Kraemer, Moritz U. G. et al. The effect of human mobility and control measures on the COVID-19 epidemic in China. *Science* **368**, 493–497 (2020).

[8] Hamada S. Badr et al. Association between mobility patterns and COVID-19 transmission in the USA: a mathematical modelling study. *Lancet Infect. Dis.* **20**, 1247–54 (2020).

[9] Leung, K., Wu, J.T. and Leung, G.M. Real-time tracking and prediction of COVID-19 infection using digital proxies of population mobility and mixing. *Nat. Commun.* **12**, 1501 (2021).

[10] Sulyok, M., and Walker, M. Community movement and COVID-19: A global study using Google's Community Mobility Reports. *Epidemiology and Infection,* **148**, E284 (2020).

[11] Athanasios A., Irini F., Tasioulis T., Konstantinos, K. Prediction of the effective reproduction number of COVID-19 in Greece. A machine learning approach using Google mobility data. medRxiv 2021.05.14.21257209; https://doi.org/10.1101/2021.05.14.21257209

[12] Nouvellet, P., Bhatia, S., Cori, A. et al. Reduction in mobility and COVID-19 transmission. *Nat. Commun.* **12**, 1090 (2021).

[13] Unwin, H.J.T., Mishra, S., Bradley, V.C. et al. State-level tracking of COVID-19 in the United States. *Nat. Commun.* **11**, 6189 (2020).

[14] Milman, O., Yelin, I., Aharony, N. et al. Community-level evidence for SARS-CoV-2 vaccine protection of unvaccinated individuals. *Nat Med* **27**, 1367–1369 (2021).

[15] Pritchard, E., Matthews, P.C., Stoesser, N. et al. Impact of vaccination on new SARS-CoV-2 infections in the United Kingdom. *Nat. Med.* **27**, 1370–1378 (2021).

[16] Google Mobility Reports. See how your community is moving around differently due to COVID-19. Accessed July 29, 2021.

[17] Jara, A. et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *New England Journal of Medicine,* **385**, 875–884, (2021).

[18] Cerqueira-Silva T. et al. Influence of age on the effectiveness and duration of protection in Vaxzevria and CoronaVac vaccines. medRxiv 2021.08.21.21261501. https://doi.org/10.1101/2021.08.21.21261501

[19] Sauré D. et al. Dynamic IgG seropositivity after rollout of CoronaVac and BNT162b2 COVID-19 vaccines in Chile: a sentinel surveillance study. *The Lancet, Infectious Diseases*, September 09, 2021. https://doi.org/10.1016/S1473-3099(21)00479-5

[20] Ministerio de Salud. Argentina. Datos Abiertos del Ministerio de Salud. Accessed 2021-08-27.

[21] Ministério de Saude. Brasil. Covid-19 Vacinaçao. Doses Aplicadas. Accessed 2021-08-26.

[22] Ministerio de Salud. Chile. Vacunación SARS-Cov-2. Accessed 2021-08-26.

[23] C. E. Schaerer. Personal communication, 2021-08-24.

[24] Ministerio de Salud Pública. Uruguay. Catálogo de datos abiertos. Accessed 2021-08-26.

[25] Rossman, H., Shilo, S., Meir, T. et al. COVID-19 dynamics after a national immunization program in Israel. *Nat. Med.* **27**, 1055–1061 (2021).

[26] Omer S. B., Yildirim I, Forman H. P. Herd Immunity and Implications for SARS-CoV-2 Control. *JAMA* **324**, 2095–2096 (2020). doi:10.1001/jama.2020.20892

[27] Marcus Carlsson, Cecilia Söderberg-Nauclér. Indications that Stockholm has reached herd immunity, given limited restrictions, against several variants of SARS-CoV-2 medRxiv 2021.07.07.21260167; https://doi.org/10.1101/2021.07.07.21260167

[28] M. Gabriela et al. Individual variation in susceptibility or exposure to SARS-CoV-2 lowers the herd immunity threshold medRxiv 2020.04.27.20081893. https://doi.org/10.1101/2020.04.27.20081893

[29] Tom Britton, Frank Ball, and Pieter Trapman. A mathematical model reveals the influence of population heterogeneity on herd immunity to SARS-CoV-2. *Science*, **369** 6505, 846–849 (2020).

[30] Infección por COVID 19: estudio seroepidemiológico de cohorte de base poblacional estratificado por edad en Asunción y Central. Sequera G. et al. http://dgvs.mspbs.gov.py/files/img/archivos/Estudio_seroepidemiologico.pdf Accessed 2021-09-01.

[31] Miraglia J.L., et al. A seroprevalence survey of anti-SARS-CoV-2 antibodies among individuals 18 years of age or older living in a vulnerable region of the city of São Paulo, Brazil. *PLoS ONE* **16**, (7): e0255412 (2021).

[32] Ministerio de Ciencia, Tecnología, Conocimiento, e Innovación de Chile. https://github.com/MinCiencia/Datos-COVID19/blob/master/output/producto83/vacunacion_fabricantes_1eraDosis.csv Accessed 07-23-2021.

[33] Al Kaabi N. et al. Effect of 2 Inactivated SARS-CoV-2 Vaccines on Symptomatic COVID-19 Infection in Adults: A Randomized Clinical Trial. *JAMA*, **326**, 35–45 (2021).

[34] Dagan, N. et al. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *New England Journal of Medicine*, **384**, 1412–1423 (2021).

[35] Stephen J. T., et. al. Six Month Safety and Efficacy of the BNT162b2 mRNA COVID-19 Vaccine. medRxiv 2021.07.28.21261159; https://doi.org/10.1101/2021.07.28.21261159

[36] Anderson, R. M., Vegvari, C., Truscott, J., Collyer, B. S., Challenges in creating herd immunity to SARS-CoV-2 infection by mass vaccination. *The Lancet* Comment volume **396** 1614–1616 (2020).

[37] Brüningk, S. C. et al. Determinants of SARS-CoV-2 transmission to guide vaccination strategy in an urban area. medRxiv 2020.12.15.20248130. https://doi.org/10.1101/2020.12.15.20248130

[38] Hjorleifsson, K. E. et al. Reconstruction of a large-scale outbreak of SARS-CoV-2 infection in Iceland informs vaccination strategies. medRxiv 2021.06.11.21258741.

[39] Aschwanden, C. Why herd immunity for covid is probably impossible. *Nature*, **591**, 520–522 (2021).

[40] Pouwels, K. B. et al. Impact of Delta on viral burden and vaccine effectiveness against new SARS-CoV-2 infections in the UK. medRxiv 2021.08.18.21262237. https://doi.org/10.1101/2021.08.18.21262237

[41] Goldberg Y. et al. Waning immunity of the BNT162b2 vaccine: A nationwide study from Israel. medRxiv 2021.08.24.21262423. https://doi.org/10.1101/2021.08.24.21262423

[42] Israel, A. et al. Elapsed time since BNT162b2 vaccine and risk of SARS-CoV-2 infection in a large cohort medRxiv 2021.08.03.21261496. https://doi.org/10.1101/2021.08.03.21261496

[43] Mizrahi B. et al. Correlation of SARS-CoV-2 Breakthrough Infections to Time-from-vaccine; Preliminary Study. medRxiv 2021.07.29.21261317. https://doi.org/10.1101/2021.07.29.21261317

[44] Gazit, S. et al. Comparing SARS-CoV-2 natural immunity to vaccine-induced immunity: reinfections versus breakthrough infections. medRxiv 2021.08.24.21262415; https://doi.org/10.1101/2021.08.24.21262415

[45] Wang, Z., Muecksch, F., et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature*, **595**, 426–431 (2021).

[46] Cohen et al., 2021, *Cell Reports Medicine* **2**, 100354 (2021).

[47] Crotty, S., Hybrid immunity. *Science* **372**1392–1393, (2021).

[48] Aktay A. et al., Google COVID-19 Community Mobility Reports: Anonymization Process Description (version 1.1). November 4, (2020). arXiv:2004.04145v4 [cs.CR]

[49] Verity, R. et al., Estimates of the severity of coronavirus disease 2019: a model-based analysis. *The Lancet, Infectious Diseases*, **20**, 669–677 (2020).

[50] Population Pyramids of the World from 1950 to 2100. https://www.populationpyramid.net/. Accessed 2021-08-31.

[51] Moghadas, S. et al. Population Immunity Against COVID-19 in the United States. *Annals of Internal Medicine*, https://doi.org/10.7326/M21-2721

A Supplementary Material

In figures A.1 and A.2 we provide the same analysis shown in figures 1 and 2 in the case of Israel, respectively.

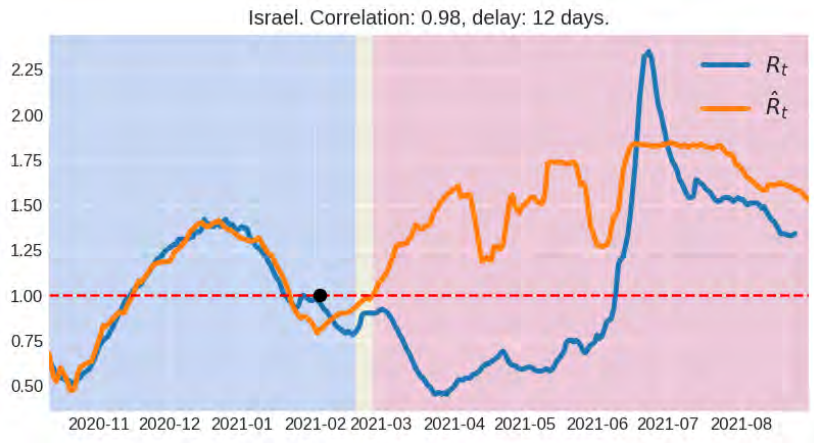


Figure A.1: Viral effective reproduction number R_t and its estimation $\hat{R}_t$ using mobility information. Background colors indicate the following time periods: in blue, the time period used to fit the linear model (see Section 4.2), in yellow, the period after the fitting, but before the decoupling point, and in red after the decoupling point. The black dot corresponds to the last time the reproductive number was above one. The correlation corresponds to the period used to fit the model. The delay indicated is the time-shift between the mobility time series and R_t in order to maximize the correlation in the linear regression.

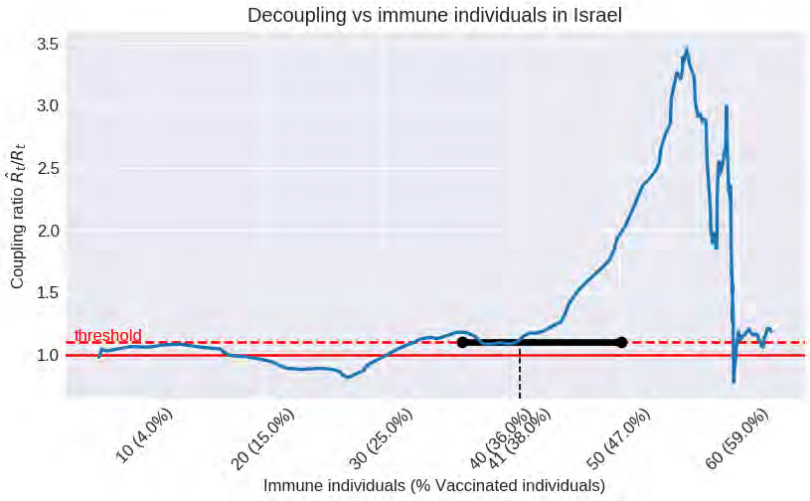


Figure A.2: Coupling ratio $\hat{R}_t/R_t$ plotted with respect to the percentage of immune population. During the first months of 2021 the coupling ratio varies around 1, which corresponds to the periods where the R_t and $\hat{R}_t$ are in concordance in Figure A.1. Immune population includes immunity achieved by vaccination (taking into account its effectiveness), and natural infection (see subsection 4.3). The percentage of people fully vaccinated is described as well.

CoronaVac

O que a ciência comprova

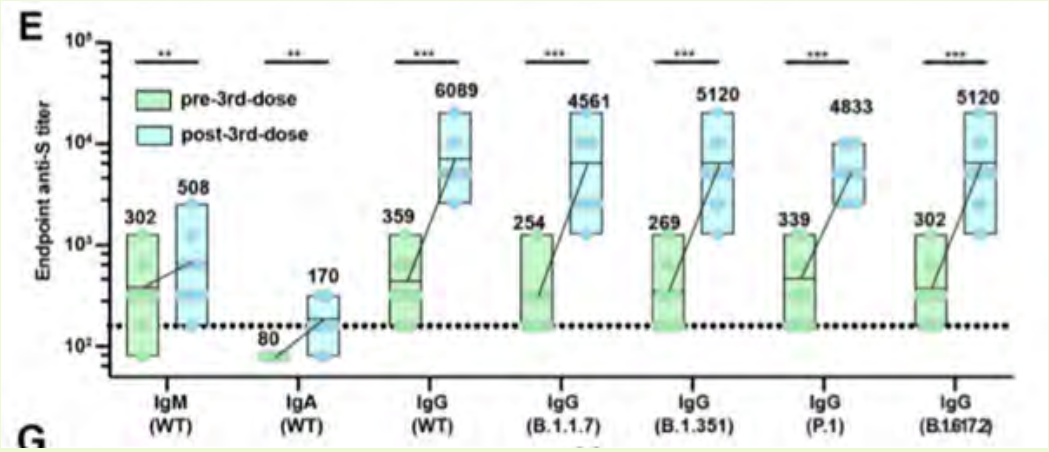
2.8. Dose de reforço da CoronaVac eleva em 17 vezes os níveis de anticorpos capazes de combater a variante delta do SARS-CoV-2, aponta estudo

Uma dose de reforço da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, aumenta em 17 vezes o nível de anticorpos neutralizantes contra a variante delta do vírus SARS-CoV-2 em quem já completou o esquema vacinal há seis meses. As conclusões estão no estudo A third dose of inactivated vaccine augments the potency, breadth, and duration of anamnestic responses against SARS-CoV-2, de pesquisadores da Academia Chinesa de Ciências, Universidade de Pequim, Faculdade de Medicina de Xangai e Sinovac, entre outras instituições, publicado na plataforma de preprints MerRxiv.

O estudo apontou que a dose de reforço da CoronaVac potencializa rapidamente e de forma robusta os níveis de anticorpos neutralizantes contra a proteína S, componente que o SARS-CoV-2 usa para invadir células humanas. Além de aumentar em 17 vezes a proteção contra a delta, a dose de reforço aumenta em 17 vezes o nível de anticorpos neutralizantes contra o vírus original (cepa de Wuhan); em 18 vezes contra a variante alfa; em 19 vezes contra a beta; e em 14 vezes contra a gama.

A pesquisa analisou amostras de plasma de 66 participantes, incluindo 38 voluntários que receberam duas ou três doses da vacina. A avaliação aconteceu quatro semanas após a administração da dose de reforço, sendo que esta foi aplicada seis meses após os indivíduos receberem a segunda dose.

O gráfico mostra o aumento do nível de anticorpos dos participantes da pesquisa, medidos imediatamente antes de tomarem a dose de reforço da CoronaVac (em verde), e passadas quatro semanas após a dose de reforço (em azul). São exibidos os resultados para o vírus original de Wuhan (WT, sigla para "wide type"), e para cada uma das quatro variantes de preocupação: alfa (B.1.1.7), beta (B.1.351), gama (P.1) e delta (B.1.617.2).



A CoronaVac já se mostrou eficaz contra a variante gama no estudo de efetividade Projeto S, realizado pelo Butantan no município paulista de Serrana. Por meio dele, 95% da população adulta foi vacinada com CoronaVac entre fevereiro e abril de 2021, quando a variante gama já era predominante no Brasil. A imunização coletiva fez os óbitos por Covid-19 despencarem 95%, as internações, 86%, e os casos sintomáticos, 80%.

Outra pesquisa realizada na China já apontava a eficácia da CoronaVac contra a variante delta. Um estudo do Centro de Controle e Prevenção de Doenças da província de Guangdong, feito durante um surto de Covid-19 causado pela delta, mostrou que a CoronaVac evitou o desenvolvimento de casos graves de Covid-19 e teve eficácia de 69,5% contra o aparecimento de pneumonias decorrentes da doença. O estudo envolveu 10.813 pessoas e foi realizado entre maio e junho de 2021

Publicado em: 5/09/2021

A third dose of inactivated vaccine augments the potency, breadth, and duration of anamnestic responses against SARS-CoV-2

Authors: Kang Wang[1$], Yunlong Cao[3$], Yunjiao Zhou[2$], Jiajing Wu[4,$], Zijing Jia[1$], Yaling Hu[5$], Ayijiang Yisimayi[3], Wangjun Fu[1], Lei Wang[1], Pan Liu[1], Kaiyue Fan[1], Ruihong Chen[1,6], Lin Wang[5], Jing Li[5], Yao Wang[3], Xiaoqin Ge[5], Qianqian Zhang[2], Jianbo Wu[2], Nan Wang[1], Wei Wu[2], Yidan Gao[2], Jingyun Miao[7], Yinan Jiang[7], Lili Qin[7], Ling Zhu[1], Weijin Huang[5], Yanjun Zhang[9], Huan Zhang[8], Baisheng Li[8], Qiang Gao[5], Xiaoliang Sunney Xie[3]*, Youchun Wang[4]*, Qiao Wang[2]* and Xiangxi Wang[1,6]*

Affiliation:

[1] CAS Key Laboratory of Infection and Immunity, National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

[2] Key Laboratory of Medical Molecular Virology (MOE/NHC/CAMS), School of Basic Medical Sciences; Shanghai Institute of Infectious Disease and Biosecurity; Shanghai Medical College, Fudan University, Shanghai 200032, China

[3] Beijing Advanced Innovation Center for Genomics (ICG), Biomedical Pioneering Innovation Center (BIOPIC), School of life Science, Peking University, Beijing 100091, China

[4] Division of HIV/AIDS and Sex-transmitted Virus Vaccines, Institute for Biological Product Control, National Institutes for Food and Drug Control (NIFDC), Beijing 102629, China

[5] Sinovac Biotech Ltd, Beijing, China

[6] Guangzhou Laboratory, Guangzhou, Guangdong, China, 510320

[7] Acrobiosystems Inc, Beijing, China

[8] Guangdong Provincial Center for Disease Control and Prevention

[9] Department of Microbiology, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China

*Correspondence to: X.W. (Email: xiangxi@ibp.ac.cn) or Q.W. (Email: wangqiao@fudan.edu.cn) or Y.W. (Email: wangyc@nifdc.org.cn) or X.S.X. (Email: sunneyxie@biopic.pku.edu.cn)

[$] These authors contributed equally to this work.

Abstract: (~150 words)

Emergence of variants of concern (VOC) with altered antigenic structures and waning humoral immunity to SARS-CoV-2 are harbingers of a long pandemic. Administration of a third dose of an inactivated virus vaccine can boost the immune response. Here, we have dissected the immunogenic profiles of antibodies from 3-dose vaccinees, 2-dose vaccinees and convalescents. Better neutralization breadth to VOCs, expeditious recall and long-lasting humoral response bolster 3-dose vaccinees in warding off COVID-19. Analysis of 171 complex structures of SARS-CoV-2 neutralizing antibodies identified structure-activity correlates, revealing ultrapotent, VOCs-resistant and broad-spectrum antigenic patches. Construction of immunogenic and mutational heat maps revealed a direct relationship between "hot" immunogenic sites and areas with high mutation frequencies. Ongoing antibody somatic mutation, memory B cell clonal turnover and antibody composition changes in B cell repertoire driven by prolonged and repeated antigen stimulation confer development of monoclonal antibodies with enhanced neutralizing potency and breadth. Our findings rationalize the use of 3-dose immunization regimens for inactivated vaccines.

One sentence summary

A third booster dose of inactivated vaccine produces a highly sifted humoral immune response *via* a sustained evolution of antibodies capable of effectively neutralizing SARS-CoV-2 variants of concern.

Main Text:

The ongoing coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has lasted for one and a half years, resulting in an unprecedented public health crisis with over 4 million deaths globally. Progress in halting this pandemic seems slow due to the emergence of variants of concern (VOC), such as the B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma, also known as B.1.1.28.1) and more recent B.1.617.2 (Delta), that appear to be high transmissible and more resistant to neutralizing antibodies *(1-4)*. While several types of COVID-19 vaccines are being deployed at a large scale, new variants are thought to be responsible for re-infections, either after natural infection or after vaccination, as observed in Brazil and the United States, respectively *(5, 6)*. Closely correlated with these, a general decrease in immune protection against SARS-CoV-2 variants within 6-12 months after the primary infection or vaccination is also observed *(6-8)*. The prospect of genetic recombination and antigenic drift in recent SARS-CoV-2 variants together with non-uniform immune protections arising from heterogeneously waning humoral immunity in COVID-19 convalescent or vaccinated individuals, point to the potential risks of a long-term pandemic that could endanger the global human health, diminishing social, economic and outdoor leisure activities. A plausible approach to solving this problem is the administration of a third dose of the vaccine somewhere between 6 and 12 months after the 2nd dose of vaccination for enhancing and prolonging the protection. However, not much is known about the immunogenic features of such a booster dose of a COVID-19 vaccine. In addition, there are large gaps in our understanding about correlating immunogenic findings from surrogate endpoints to gauge vaccine efficacy.

The CoronaVac, a 2-dose β-propiolactone-inactivated vaccine against COVID-19, has been approved for emergency use by the World Health Organization *(9, 10)*. In human clinical trials (phase I/II, registration number: NCT04352608), a subgroup with a 3-dose immunization schedule at months 0, 1, 7 was also included. To evaluate immune features, we recruited 22 COVID-19 convalescents, 6 healthy participants (SARS-CoV-2 negative, confirmed by RT-PCR) and 38 volunteers who received

either 2 or 3 doses of the Coronavac vaccine for blood donation. The volunteers ranged from 16 to 69 years old (median 33); 30 (45.5%) were men and 36 (54.5%) were women. None of the volunteers recruited for vaccination was infected by SARS-CoV-2 prior to the study. Blood samples from convalescents and vaccinees collected 1.3 months after infection and the indicated times after vaccination were used in this study, respectively, to compare humoral immune responses elicited against circulating SARS-CoV-2 variants.

Neutralizing antibodies (NAbs) are a major correlate of protection for many viruses, including SARS-CoV-2, and have also provided the best correlate of vaccine efficacy. Several types of SARS-CoV-2 neutralization assays have been described using either live SARS-CoV-2 or a pseudo-typed reporter virus carrying SARS-CoV-2 spike protein (S). Both types of assays could yield reproducible neutralizing titers, with the pseudo-typed virus neutralization assay exhibiting higher sensitivity (*11, 12*). Neutralizing activity of plasma samples from 66 participants was measured against WT, B.1.351, P.1 and B.1.617.2 using live SARS-CoV-2 and VSV-pseudoviruses with the S from WT, B.1.1.7, P.1 variants and SARS-CoV (Fig. 1). The geometric mean half-maximal neutralizing titers (GMT NT_{50}) against live SARS-CoV-2 in plasma obtained from convalescents and from vaccinees (4 weeks after the final vaccination) suggest an approximately 60% higher neutralizing activity against WT after 3-dose inoculation when compared with 2-dose administration, and 20% higher than those from convalescents (Fig. 1A). Interestingly, for the samples from the convalescents, 2-dose and 3-dose vaccinees, neutralizing titers against B.1.351 were, on average, 7.7-fold, 5.7-fold and 3.0-fold reduced, respectively, compared with WT (Fig. 1A). Similarly, fold decreases in neutralization ID_{50} titers against P.1 and B.1.617.2 for the three cohorts were 5.3, 4.3 and 3.1, and 5.3, 3.7 and 2.3, respectively (Fig. 1A). Overall, plasma of the 3-dose vaccinees displayed minimal reduction in neutralization titers against several authentic VOCs compared to the convalescents and 2-dose vaccinees (Fig. 1A). Remarkably, ~41% (9/22) and 50% (6/12) samples from the convalescents and 2-dose vaccinees, respectively, failed to reach 50% neutralization at a plasma dilution

of 1: 10, with ~14% (3/22) and 16% (2/12) showing a near ineffectiveness in neutralizing B.1.351 *in vitro* (Fig. 1A). By contrast, only 1 out of 14 samples from the 3-dose vaccinees exhibited a weak neutralizing titer below 10 (Fig. 1A). Importantly, the 3-dose vaccinees showed over 2.5-fold higher neutralizing potency against B.1.617.2 than the convalescents and 2-dose vaccinees (Fig. 1A). The GMT NT_{50} values measured by a VSV-pseudovirus with the WT S were 840, 660 and 1,176 for convalescents, 2-dose and 3-dose vaccinees, respectively, which were 8-10-fold greater than those determined by live WT SARS-CoV-2 (Fig. 1A, 1B), confirming higher sensitivity of pseudovirus-based assays in determining neutralizing titers. In line with the results of live SARS-CoV-2 neutralization assay, the mean fold decrease in the neutralization of B.1.1.7 relative to the WT was 2.8-fold for convalescents, 2.2-fold for 2-dose vaccinees and 1.7-fold for 3-dose vaccinees (Fig. 1B). Similarly, plasma from convalescents, 2-dose and 3-dose vaccinees exhibited a 4.5-fold, 2.9-fold and 2.4-fold reduction, in NAb titers against P.1, respectively, when compared to the WT (Fig. 1B). These results reveal that a third-dose boost of inactivated vaccine leads to enhanced neutralizing breadth to SARS-CoV-2 variants, bolstering the potential to ward off VOCs effectively when compared to convalescent plasma. Of note, neither vaccination nor SARS-CoV-2 infection boosts distinct neutralizing potency against SARS-CoV, presumably due to the relatively far phylogenic relationship (Fig. 1B).

To seek information on potential binding-neutralization correlates, the abilities of antibodies present in plasma to bind the receptor-binding domain (RBD), N-terminal domain (NTD), S-trimer and nucleoprotein (N) from SARS-CoV-2 and its variants were measured by enzyme-linked immunosorbent assay (ELISA). As expected, all COVID-19 convalescents and vaccinees exhibited high anti-RBD, anti-NTD, anti-S and anti-N titers for SARS-CoV-2 variants, but weak antibody reactivity to SARS-CoV (Fig. 1C and fig. S1). Unexpectedly, the amount of N-specific IgG elicited by 2-dose and 3-dose vaccination schedules was 2-6-fold lower than those of convalescents, and 2-6-fold lower than the antibodies targeting S or RBD in vaccinees, reflecting distinct serological profiles (Fig. 1C and fig. S1). Overall

plasma neutralizing activity against the WT was substantially correlated with anti-S and anti-RBD binding titers in ELISA. However, only marginal correlates between binding and neutralization potency were established for VOCs (fig. S2). In spite of this, a 3-dose administration elicits a broader range of antibody binding activities to VOCs with minimal decreasing folds than those of 2-dose vaccination and convalescents (Fig. 1D and fig. S2).

To evaluate the nature of humoral immune response elicited by a booster dose of CoronaVac, the S-specific IgA, IgM and IgG titers and neutralizing activities against SARS-CoV-2 variants were monitored before and 4 weeks after the third immunization. S-specific IgM and IgA titers were generally lower and were not significantly boosted in response to the third-dose vaccination (Fig. 1E). Similar to most convalescents (*2*), approximately 80~90% of both anti-S IgG and NAb titers against the WT waned 6 months after the second vaccination (*13*), while the third-dose administration of CoronaVac boosted these titers by ~20-fold at 4 weeks post vaccination (Fig. 1E and F). Significantly, vaccinees 6 months after the second immunization did not have detectable *in vitro* neutralizing activities against B.1.351, P.1 and B.1.617.2, while all vaccinees exhibited a robust recall humoral response to efficiently neutralize circulating variants post the third-dose vaccination (Fig. 1E and F). To further characterize the expeditiousness, longevity and immunological kinetics of recall response stimulated by the third-dose immunization, neutralizing potencies at days 0, 7, 14, 28, 90 and 180 post the third-dose vaccination were determined (Fig. 1G and H). Remarkably, NAb titer surged by ~8-fold (from 7 to 53) at week 1, peaked by ~25-fold increase (up to 177) at week 2 after the 3rd-booster and slowly decreased over time (Fig. 1G). Notably, NAb titer was maintained at around 60 on 180 days post the 3rd-booster, comparable to the high level of NAb titer elicited by the 2-dose administration (Fig. 1H). Taken together, these serological results reveal a third-dose booster can elicit an expeditious, robust and long-lasting recall humoral response.

The molecular mechanism underlying these potent, broad and durative antibody

responses elicited by a third-dose booster 6 months after the administration of the second dose of the vaccine, might involve ongoing antibody somatic mutation and evolution of antibody by affinity maturation through prolonged and repeated antigen stimulation *(14, 15)*. Although circulating antibodies derived from plasma cells wane over time, long-lived immune memory can persist in expanded clones of memory B cells *(16)*. Thereby, we used flow cytometry to sort the SARS-CoV-2 S-trimer-specific memory B cells from the blood of seven selected CoronaVac vaccinees, including four samples from 3-dose vaccinees and three samples from 2-dose vaccinees (Fig. 2A and fig. S3). The averaged percentage of S-binding memory B cells in 3-dose vaccinees was substantially greater than those in 2-dose vaccinees (Fig. 2A and fig. S3). Due to differences in labeling strategies employed for sorting SARS-CoV-2-specific B cells, the above percentage of memory B cells was not directly comparable with those reported in naturally infected individuals and in mRNA vaccinated individuals. The gated double-positive cells were single cell sorted and immunoglobulin heavy (*IGH*; IgG isotype) and light (*IGL* or *IGK*) chain genes were amplified by nested PCR. Overall, we obtained 422 and 132 paired heavy and light chain variable regions from S-binding IgG^+ memory B cells from four 3-dose and three 2-dose vaccinees, respectively (Fig. 2B and fig. S4). Surprisingly, expanded clones of cells comprised 45-61% of the overall S-binding memory B compartment in 3-dose vaccinees, which is approximately 2-fold higher than those in COVID-19 convalescents and in mRNA or 2-dose vaccinated individuals (Fig. 2B and C). When compared to 2-dose vaccinees, the increase in the number of persistent clones and various clonal compositions in 3-dose vaccinated group suggested an ongoing clonal evolution (Fig. 2B and C). Shared antibodies with the same combination of *IGHV* and *IGLV* genes in 3-dose vaccinees comprised ~20% of all the clonal sequences. Similar to natural infection and mRNA vaccination *(2, 14, 16)*, *IGHV3-30*, *IGHV3-53* and *IGHV1-69* remained significantly over-represented in 3-dose vaccinees (fig. S5). Meanwhile, notable differences in the frequency of human V genes between 3-dose vaccinated and the other two groups were observed as well (fig. S5). In 3-dose vaccinees, *IGHV3-21*, *IGHV4-39* and *IGHV7-4-1* were largely abundant, but *IGHV5-51*, *IGHV3-66* and *IGHV1-2* were significantly scarce when

compared to the other two groups (fig. S5), indicative of memory B cell clonal turnover. Notably, large-scale, single-cell sequencing datasets generated from two cohorts of 2-dose, 3-dose vaccinees and a group of convalescents revealed no distinct preference in the frequency of *V* genes at total B cell repertoire level (fig. S6), suggesting that a large abundance of antibodies with low expression or affinities exist in B cells. Additionally, the number of nucleotide mutations in the *V* gene in 3-dose vaccinees is higher than those in both 2-dose vaccinees and naturally infected individuals assayed after 1.3 and 6.2 months, but slightly lower than those in convalescent individuals 1 year after infection (Fig. 2D), revealing ongoing somatic hypermutation of antibody genes. There was no significant difference in the length of the IgG CDR3 between vaccinated (either mRNA or inactivated) and convalescent (after 1.3 or 6.2 or months) groups (fig. S7). These results reveal that a third-dose booster 6 months after the second vaccination elicits an enhanced and anamnestic immune response, which is led by clonal evolution of memory B cell and ongoing antibody somatic mutations, resulting in enhanced neutralizing potency, breadth and longevity of the immune response against SARS-CoV-2.

To further explore the immunogenic characteristics of the antibodies obtained from memory B cells in 3-dose vaccinees, 48 clonal antibodies, designated as XGv01 to XGv50 (no expression for XGv37 and XGv48) were expressed and their antigen binding abilities verified by ELISA (fig. S8). Biolayer interferometry affinities (BLI) measurements demonstrated that all antibodies bound to WT SARS-CoV-2 at sub-nM levels (fig. S9 and table S1). The normalized geometric mean ELISA half-maximal concentration (EC_{50}) revealed that all antibodies (EC_{50}=4.5 ng/ml) obtained from 3-dose vaccinees, in particular RBD-specific mAbs (EC_{50}=3.5 ng/ml), possessed higher binding activities than RBD-mAbs from early convalescents (at 1.3 and 6.2 months after infection, EC_{50}=5.0 and 6.8 ng/ml, respectively) and mRNA (EC_{50}=4.4 ng/ml) vaccinated individuals *(2, 14-18)*, but were comparable to those from late convalescent individuals (EC_{50}=2.6 ng/ml) assessed at 12 months after infection (Fig. 2E). These results indicate the possibility of the loss of antibodies with low binding affinities over time or an ongoing increase in affinity under the

repeated exposures of antigen. Among these antibodies tested, 26 bound to RBD, 16 targeted NTD, and 6 interacted with neither RBD nor NTD, but bound S1 (S1/non-RBD-NTD) (fig. S9 and table S1). Pseudovirus neutralization assay revealed that all RBD-specific antibodies, 10 (~60%) of the 16 NTD-directed antibodies and 3 (~50%) of the 6 S1/non-RBD-NTD antibodies were neutralizing, presenting a relatively high ratio for NAbs (Fig. 2F, fig. S10 and table S2). Authentic SARS-CoV-2 neutralization assay results largely verified their neutralizing activities, albeit with that higher concentrations were required for some NAbs (fig. S11). Compared to RBD antibodies, many NTD NAbs exhibited very limited neutralizing activities. Notably, approximately 30% of RBD antibodies showed extra potent activities with half-maximal inhibitory concentration values (IC_{50}) < 0.1 nM. In line with binding affinity, the normalized geometric mean IC_{50} of the RBD antibodies of 3-dose vaccinees was 80 ng/ml, substantially lower than those from naturally infected individuals (ranging from 1.3 to 6.2 months, IC_{50}=130-160 ng/ml) and mRNA vaccinated individuals (IC_{50}=150 ng/ml), but similar to those from late convalescents (IC_{50}=78 ng/ml) (Fig. 2E) (*2, 14-18*). The overall increased neutralizing potency might have resulted from the ongoing accumulation of clones expressing antibodies with tight binding and potent neutralizing activities. Our experimental observations are consistent with a more recent study where antibodies generated from clonal B cells after 12 months showed enhanced neutralizing activities (*14, 15*).

To examine the cross-reactivity against VOCs and other human coronaviruses, binding responses of these antibodies to WT, B.1.1.7, P.1, B.1.351, B.1.617.2, SARS-CoV, HuCoV NL63, HuCoV 229E and HuCoV HKU1 were measured. All but 2 of the 48 antibodies showed strong cross-binding to SARS-CoV-2 VOCs and about one-third of antibodies exhibited clear cross-reactivity to SARS-CoV, but none of these bound to HuCoV NL63, HuCoV 229E or HuCoV HKU1 (fig. S12). For ~ 20% and 25% of RBD- and NTD-targeting antibodies, respectively, binding affinities against B.1.351/B.1.617.2 were over 10-fold reduced compared with WT (Fig. 2E). To further determine the neutralization breadth, the neutralizing activity of these antibodies was assayed against five VOCs and SARS-CoV. Out of 26 RBD NAbs,

24 possessed cross-neutralization activity against all five SARS-CoV-2 VOCs (Fig. 2F and fig. S13). Among these, six RBD antibodies could cross-neutralize SARS-CoV, of which 2 exhibited more potent neutralization activity against SARS-CoV with IC_{50} values of 41 and 73 ng/ml. However, most of the NTD and S1/non-RBD-NTD NAbs lost their abilities to inhibit viral infection (Fig. 2F and fig. S13), indicative of higher variations for the NTD in VOCs. In comparison with NAbs from early convalescents, antibodies isolated from 3-dose vaccinees showed overall enhanced neutralizing potency and breadth to VOCs.

RBD is one of the main targets of neutralization in SARS-CoV-2 and other coronaviruses. Due to its inherent conformational flexibility, RBD exists in either an "open" (ACE2 receptor accessible) or "closed" (ACE2 receptor inaccessible) configuration *(19, 20)*, bearing antigenic sites with distinct "neutralizing sensitivity". To dissect the nature of the epitopes of RBD targeted by NAbs, 171 SARS-CoV-2 RBD-targeting NAbs with available structures *(2, 15, 21-82)*, including 8 cryo-EM structures determined in this manuscript (fig. S14-S15 and table S3), were examined. By using cluster analysis on epitope structures, the antibodies were primarily classified into six sites (I, II, III, IV, V and VI) (Fig. 3A and fig. S16), that are related to the four or five classes assigned in recent studies *(22, 31)*. Additionally, we superimposed structures of RBDs from these complex structures and calculated the clash areas between any 2 NAbs (Fig. 3B). Both strategies yielded identical results. Combining the results of the characterization of binding and neutralization studies reported previously with those determined here, the key structure-activity correlates for the six classes of antibodies were analyzed (Fig. 3). Antibodies with sites I, II and III, most frequently elicited by SARS-CoV-2 early infection, target the receptor-binding motif (RBM), and potently neutralize the virus by blocking the interactions between SARS-CoV-2 and ACE2 (Fig. 3C and D). Class I antibodies, mostly derived from *IGHV3-53/IGHV3-66* with short HCDR3s (generally <15 residues), recognize only the "open" RBD, and make contact with K417 and N501, but not L452/T478/E484 (Fig. 3C and D, and fig. S16-S17). Notably, mutations such as K417N, L452R, T478K, E484K and N501Y, or combinations of these mutations,

identified in several VOCs like B.1.1.7, B.1.617.2, P.1 and B.1.351, have been demonstrated to be key determinants for the viral escape of neutralization by many NAbs (fig. S18) (*1, 81*). Approximately ~75% and 60% of class I NAbs were significantly impaired in binding and neutralizing activities against B.1.351 as well as P.1, respectively, due to the combined mutations of K417N/T and N501Y (Fig. 3D and E, and fig. S18). Contrarily, Class III antibodies that are encoded by *IGHV1-2* and other variable heavy (VH)-genes and bound to RBD either in "open" or "closed" conformation, extensively associate with E484, and partially with L452, but not K417/T478/N501 (Fig. 3D and fig. S17C). Interestingly, *IGHV3-53/IGHV3-66* RBD antibodies with long HCDR3s (>15 residues) switch their epitopes from the site I to site III, indicating a clear antigenic drift during the process of somatic hypermutations (fig. S17C). Disastrously, over 90% class III antibodies showed a complete loss of activity against B.1.351 as well as P.1 largely owing to an E484K mutation (Fig. 3E). Against B.1.617.2, the substantially decreased activity of ~half of the class III antibodies is presumably mediated by L452R (Fig. 3E). Class II antibodies use more diverse VH-genes and target the patch lying between sites I and III (Fig. 3D and fig. S19). Surprisingly, antibodies binding to site II possess relatively lower specificity in recognition of epitope clusters ranging from K417, L452, S477, E484 to N501 (fig. S16). Like site I, site II can only be accessed when the RBD is in "open" conformation (Fig. 3A). As expected, the effects of mutations on the activity of class II antibodies were severe, two-thirds of these antibodies had >10-fold fall in neutralization activities against VOCs (Fig. 3E). Overall, the above analysis reveals that the RBD mutations identified in several VOCs can significantly reduce and, in some cases, even abolish the binding and neutralization of classes I to III antibodies, albeit being the most potent neutralizing antibodies against WT SARS-CoV-2.

By contrast, antibodies of the other three classes recognize evolutionarily conserved regions distinct from the RBM and some of these are often cross-reactive with other sarbecoviruses (*65-67, 79*). The binding of class IV antibodies, albeit attached to the apical shoulder of the RBM, is focused on a condensed patch that comprises residues 345-346, 440-441, 444-446, 448-450, which are not related to mutations observed in

VOCs (Fig. 3C and fig. S16). Related to the binding position, site IV epitopes, accessible in both "open" and "closed" conformations, exist either as partially overlapped with or outside ACE2 binding sites (Fig. 3A). Interestingly, class IV antibodies can execute their neutralizations via multiple mechanisms, such as (i) direct blockage of RBD-ACE2 associations, (ii) bridging adjacent "closed" RBDs to lock the S-trimer into a completely closed prefusion conformation, (iii) blockage of viral membrane fusion by locking conformational changes of the S-trimer, or (iv) Fc-dependent effector mechanisms (*31, 62, 67*). Class IV antibodies, e.g. 1-57, 2-7, S309 and BD-812, hold the greatest potential for harboring ultra-potent neutralization activity and markedly high tolerance to most VOCs (*63, 67*). Not surprisingly, all class IV antibodies, but CV07-270, exhibited excellent neutralizing breadth and potency to VOCs (Fig. 3E). The probable reason underlying the exception could be that CV07-270 bears an unusually long HCDR3, directly contacting E484, distal to the site IV (*46*). Site V locates beneath the RBM ridge, opposite to the site I, and adjacent to the site III. None of the class V antibodies compete with ACE2 binding (Fig. 3D and fig. S17). Due to ~40% targeting frequency to L452, B.1.617.2, but not other VOCs, partially decreased the activities of some class V antibodies (Fig. 3E). Class VI antibodies recognize a patch on one side of the RBD, distal from the RBM. Among these, some compete with ACE2 binding, while some do not, and this largely depends on the orientation/pose of the antibodies bound. Both sites V and VI contain cryptic epitopes that are only accessible when at least one RBD is in the open state (Fig. 3A and C). In some cases, e.g. FC08 and CR3022, belonging to class V and VI, respectively, epitopes are only accessible in the prefusion S-trimer under the condition that all RBDs are open, suggesting that binding of these antibodies would facilitate the destruction of the prefusion S-trimer (*83, 84*). In spite of less potency, antibodies targeting sites V to VI are mostly tolerant to the VOCs.

Low levels of NAbs elicited by either natural infection or vaccination during *in vivo* viral propagation may impose strong selection pressure for viral escape, leading to an increase in the number of SARS-CoV-2 variants. To further understand the drivers of viral evolution, we constructed immunogenic and mutational heatmaps for RBD

using the 171 NAb complex structures to estimate *in vivo* NAb-targeting frequencies on the RBD and viral mutation frequencies (calculated from the datasets in the GISAID), respectively (Fig. 3D and fig. S19). Briefly, for each antibody, we identified epitope residues and calculated the frequency of each RBD residue being recognized by antibody. Immunogenic heatmap revealed that the epitope residues of sites I to III showed predominantly higher NAb recognition frequencies (about 53.8, 55.0 and 49.2 antibodies per residue on average for site I, II and III, respectively) compared with those of sites IV to VI (about 19.4, 9.1 and 14.3 antibodies per residues on average for site IV, V and VI, respectively), suggesting that class I to III antibody epitopes are "hot" immunogenic sites (Fig. 3D and fig. S19). In line with this, residues within sites I to III exhibited dramatically higher mutation frequencies, as revealed in circulating variants that include mutations of K417, L452, S477, T478, E484 and N501 residues (Fig. 3D and fig. S19). Surprisingly, none of the top 9 hottest immunogenic residues had a high mutation frequency. In particular, residues, such as F486, Y489, Q493, L455, F456, *et.al* (top 5, having 96, 96, 81, 73 and 70 antibodies per residue, respectively) with large side chains exhibited extremely low mutation frequencies in circulating SARS-CoV-2 strains (Fig. 3D and fig. S20). It's worthy to note that all these residues are extensively involved in the recognition of ACE2. The buried surface area (BSA) of these residues upon binding to ACE2 confirmed that extensive interactions would be significantly reduced by amino acid substitutions, thereby affecting ACE2-mediated viral entry. Thus, genetic, structural and immunogenic analysis explains why mutations at these positions would not be selected.

A few studies have reported that a subset of NTD-targeting antibodies can be as potent as best-in-class RBD specific antibodies. They work *via* inhibiting a step post-attachment to cells like blocking fusion of the virus to the host cell membrane *(85-88)*. We performed cluster analysis on 26 structures of the NTD-NAb complexes (including 2 structures solved in this manuscript) (fig. S21A) *(54, 85-93)*. A dominant site α, defined as the "supersite" in more recent studies *(85-88)*, comprising of three flexible loops (N1, N3 and N5), is the largest glycan-free surface of NTD

facing away from the viral membrane (facing up). Antibodies targeting site α generally exhibited the most potent neutralizing activity compared to other sites on the NTD (*85, 90*) (fig. S21B and C). The NTD supersite antibodies are primarily derived from a subset of VH-genes with an over-representation of *IGHV1-24*. Sites β and γ, as the left and right flank clusters, construct a shallow groove beneath the supersite and locate at the back of the groove, eliciting less potent antibodies. By contrast, δ antibodies, bound to a patch beneath the groove have their Fab constant domains directed downward toward the virus membrane (facing down) (fig. S21B and C). In line with binding orientation, many of the δ antibodies were shown to present infection enhancing activities *in vitro* (*54, 90*). Perhaps correlated with being a "hot" immunogenic site that is amenable to potent neutralization, highly frequent mutations, including a number of deletions within the NTD supersite were identified in most VOCs under ongoing selective pressure, leading to significant reduction and in some cases even complete loss of neutralization activity for these NTD supersite NAbs (*94*).

More recent studies have reported that SARS-CoV-2 infection can produce a long-lasting memory compartment that continues to evolve over 12 months after infection with ongoing accumulation of somatic mutations, emergence of new clones and increasing affinity of antibodies to antigens (*14, 15*). Consequently, an increase in breadth and overall potency of antibodies produced by memory B cells over time has been revealed (*14*), akin to the experimental observations elicited by a 3-dose vaccination strategy using an inactivated vaccine described in this study. To investigate whether changes in the frequency of distribution of the six types of RBD antibodies is associated with evolution time, we collated and categorized human SARS-CoV-2 NAbs from available literatures. For antibody clustering, we combined structural and square competition matrix analysis for 273 RBD NAbs in total (Fig. 4A and fig. S22). In the earliest documented studies (before Dec 2020), NAbs belonging to classes I to III were predominantly identified in early COVID-19 convalescent and 2-dose vaccinated individuals (defined as early time point), accounting for up to ~80% of total antibodies. By contrast, a low ratio of NAbs from

IV to VI was reported possibly due to their less potent activities at the early time point (Fig. 4A). In recent literatures (after Dec 2020), NAbs with enhanced neutralizing potency and breadth from IV to VI have substantially been enriched in the late convalescents or 3-dose vaccinees, almost equal in frequency to antibodies from I to III and further becoming ascendant in individuals immunized with 3 doses of inactivated vaccine (Fig. 4A). Differential frequency of distribution of antibody types may provide an additional possible explanation for the observed enhanced neutralizing breadth of plasma in late convalescent individuals and 3-dose vaccinees. These results suggest that memory B cells display clonal turnover after about 6 months, subsequently resulting in changes in the composition of antibodies in B cell repertoire and thereby partially contributing to enhanced activities of antibodies secreted in the plasma over time. To explore the underlying mechanism, we measured the binding affinities of 167 type-classified antibodies that are also further categorized into early and late time point groups (table S1 and fig. S9). For the late time group, there was a 10-20 fold increase in binding affinity for individual classes, compared to those in the early time point group (Fig. 4B). In early time point group, antibodies from IV to VI exhibited higher binding affinities to the RBD than those from I to III, in particular, antibodies from V and VI despite limited numbers (Fig. 4B). Possibly higher affinities for these antibodies are required to accomplish neutralization successfully. Thus, most antibodies from V and VI with low affinities and activities might be screened out in the early time point. In the late point group, sub-*nM* binding affinities for individual class antibodies with no distinct variations were observed, reflecting ongoing affinity maturation over time. This might also explain the observation that some antibodies, from I to III isolated in the late time point possess potent cross-neutralization activities (Fig. 3E). Our antibody clustering and *V* gene usage analysis suggests that individual class antibodies can be derived from multiple *V* genes and the shared *V* gene antibodies belong to different classes. To decipher the intrinsic trends in the relationship between binding affinity and somatic hypermutation (SHM) rate, we determined the relative affinity (K_D) and calculated the SHM rate of antibodies that are encoded by the same *V* gene and belong to the same class. The measured K_D–SHM plots and K_D–SHM log-log plots of

class I antibodies (n=61), including 32 NAbs derived from *IGHV3-53*, show least squares fitting of data to a power law with a strong correlation of -0.81 for *IGHV3-53* antibodies (-0.55 for all class I antibodies) (Fig. 4C). The absolute value of its slope corresponding to a free energy change per logarithm (base e) *SHM* of cal nmol^{-1}, where free energy change is $4.98RT + 1.48RT \ln(SHM)$ ($R = 2.0\ \text{cal K}^{-1}\ \text{nmol}^{-1}$ and $T = 298$ K). Antibodies with adequate numbers tested from II and III exhibited similar trends by following a power law, among which *IGHV3-66* antibodies in class II yielded a compelling correlation of -0.94 despite 6 plots involved in the fitting (Fig. 4C). These trends indicate that as the SHM increase, the binding energy increases and K_D value decreases.

More recently, the B.1.617.2 variant has contributed to another surge in COVID-19 cases worldwide, accounting for ~90% of new cases in the UK and >40% in the US, despite the fact that increasing number of people have been vaccinated. Evaluation of the effectiveness of several vaccines performed recently suggests that the efficacy for VOCs correlates with full vaccination status and the time that has passed since vaccination *(95, 96)*. These may indicate that the effectiveness of the vaccines has started to decline as months pass after vaccination due to fading immunity. Our results demonstrate that a third-dose booster of inactivated vaccine can elicit an expeditious, robust and long-lasting recall humoral response which continues to evolve with ongoing accumulation of somatic mutations, emergence of new clones and increasing affinities of antibodies to antigens, conferring enhanced neutralizing potency and breadth. Collectively, our findings rationalize the use of 3-dose vaccination regimens.

Acknowledgments: We thank Dr. Xiaojun Huang, Dr. Boling Zhu, Dr. Lihong Chen, Dr. Xujing Li and Dr. Gang Ji for cryo-EM data collection, the Center for Biological Imaging (CBI) in Institute of Biophysics for EM work and thank Dr. Yuanyuan Chen, Zhenwei Yang for technical help with BLI experiments. We also thank Prof. David Ho and Prof. Barton Ford Haynes for generous gift of the sequences of two reference mAbs. Work was supported by the Strategic Priority Research Program

(XDB29010000, XDB37030000), CAS (YSBR-010), National Key Research and Development Program (2020YFA0707500, 2018YFA0900801), Emergency Key Program of Guangzhou Laboratory, Grant No. EKPG21-09 and Beijing Municipal Science and Technology Project (Z201100005420017). Xiangxi Wang was supported by Ten Thousand Talent Program and the NSFS Innovative Research Group (No. 81921005). **Author contributions**: X.W., Q.W., Y.W., X.S.X and Y.C. conceived, designed and coordinated the experiments. Y.Z., A.Y., Y.W., Q.Z. and J-B.W. cloned and produced antibodies. K.W., Z.J., K.F., J.M., Y.J. and L.Q. expressed and purified all recombinant antigen proteins used in this manuscript. J-J.W., Y.H., L.W., J.L., X.G., Y-J.Z., H.Z. and B-S.L performed pseudovirus and authentic virus neutralization experiments. K.W., L.W., P. L., WJ.F, N.W and L.Z performed structural study. Y.C., Y.Z., W.W. and Y.G. prepared PBMCs and flow cytometry sorting. Y.C. and A.Y. performed 10X sequencing library construction. Z.J. and K.F performed BLI assay. Y.H. and Q.G recruited volunteers and coordinated the collection of blood samples. All authors analyzed data; X.W wrote the manuscript with input from all authors. **Competing interests**: All authors have no competing interests. **Data and materials availability**: Cryo-EM density maps of the SARS-CoV-2 S trimer in complex with XGv013 or XGv043, the SARS-CoV-2 S trimer in complex with XGv004, XGv030 and XGv016; the SARS-CoV-2 S trimer in complex with XGv026 and XGv046, and the SARS-CoV-2 S trimer in complex with XGv018, XGv038 and XGv42 have been deposited at the Electron Microscopy Data Bank with accession codes EMD-UUUU, EMD-WWWW, EMD-XXXX, EMD-YYYY and EMD-ZZZZ, respectively.

Figure legends

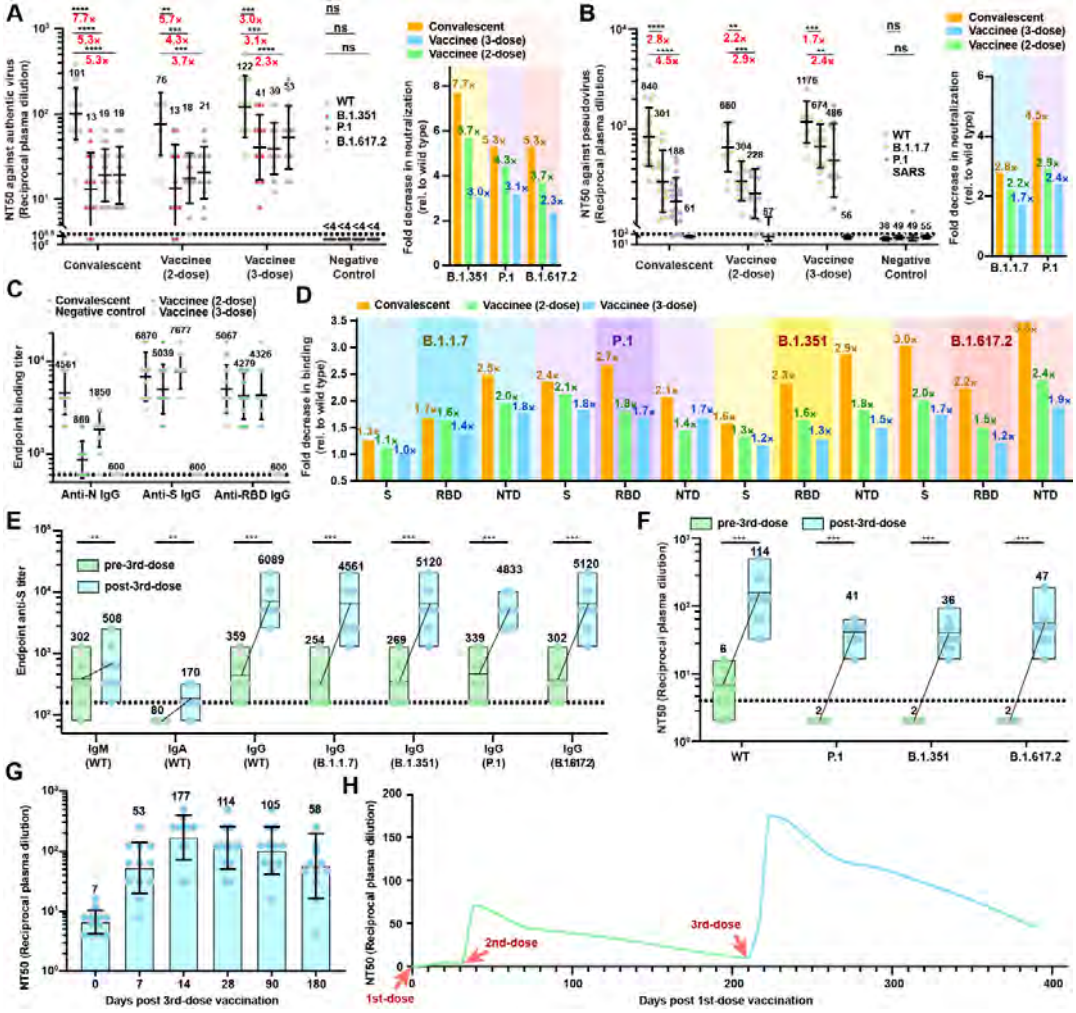


Fig. 1 A 3rd-dose booster of an inactivated vaccine elicits an expeditious and long-lasting recall antibody response

Plasma neutralizing activity evaluated by authentic SARS-CoV-2 **(A)** and pseudo-typed SARS-CoV-2 neutralization assays **(B)** Left: half-maximal neutralizing titer (NT_{50}) values for plasma from COVID-19 convalescents, 2-dose, 3-dose CoronaVac vaccine recipients (at week 4 after the last dose of vaccination) and negative controls (pre-COVID-19 historical control) against live SARS-CoV-2 WT, B.1.351, P.1 and B.1.617.2, and VSV-based SARS-CoV-2 pseudoviruses bearing WT or B.1.1.7 or P.1 S protein. Black bars and indicated values represent geometric mean NT_{50} values. Statistical significance was determined using the two-tailed Wilcoxon matched-pairs test. Experiments were repeated in triplicate. Dotted lines indicate the limit of detection. Right: fold decrease in neutralization for each variant relative to WT for

each cohort of plasma samples (calculated from the left datasets) is shown.

(C) IgG endpoint antibody responses specific to the N, RBD and S of WT SARS-CoV-2 were measured in plasma samples collected from cohorts as described earlier.

(D) Fold decrease in specific binding to the RBD, NTD and S for each variant over WT for each cohort of plasma samples as described above.

(E) IgA, IgM and IgG endpoint antibody titers specific to the S of WT SARS-CoV-2 or its variants in plasma samples collected from vaccinees before and 4 weeks after the 3rd-dose immunization.

(F) Neutralizing titers against live SARS-CoV-2 WT, P.1, B.1.351 and B.1.617.2 for plasma from vaccinees before and 4 weeks after the 3rd-dose immunization. Black bars and indicated values represent geometric mean NT_{50} values.

(G) Longitudinal neutralizing titers of plasma from 3-dose vaccinees at days 0, 7, 14, 28, 90 and 180 post the 3rd-dose vaccination. The geometric mean NT_{50} values are labeled.

(H) Kinetics of the 3rd-dose booster elicited recall response as indicated during monitoring of NAb titers at different time points. The green and blue curves show the changes in kinetics of NAb titers for pre-3rd-dose and post-3rd-dose vaccination, respectively.

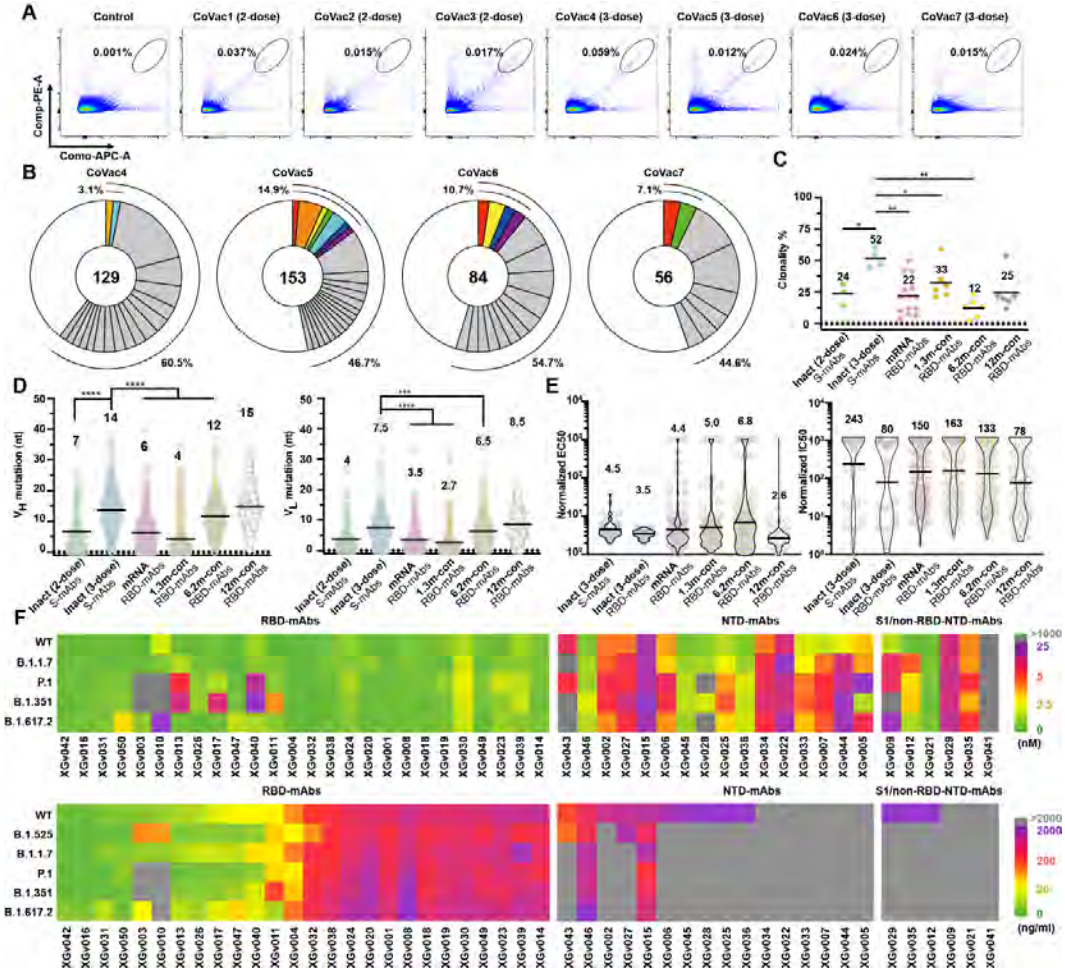


Fig. 2 Memory B cell antibodies elicited by a 3rd-dose booster of an inactivated vaccine

(A) Representative flow cytometry plots showing dual allophycocyanin (APC)-S- and phycoerythrin (PE)-S-binding B cells for vaccinees and control donor.

(B) Pie charts represent the distribution of antibody sequences from the four 3-dose vaccinees. The number in the inner circle is the number of sequences analyzed here. Pie-slice size is proportional to the number of clonally related sequences. The black outline indicates the frequency of clonally expanded sequences detected individually. Colored slices reveal clones that share the same *IGHV* and *IGLV* genes.

(C) Graph shows relative clonality among seven individuals who received 2-dose or 3-dose of inactivated vaccines. Relative clonality for COVID-19 convalescents assayed at 1.3, 6.2 and 12 months after infection, as well as 2-dose mRNA vaccine recipients (*2, 14, 18*), previously described by Michel's group, was compared. Black

582 horizontal bars indicate mean values. Statistical significance was determined using
583 two-tailed t-test.
584 **(D)** Number of somatic nucleotide mutations in the *IGHV* (left) and *IGLV* (right) in
585 antibodies from vaccinees, including 2-dose or 3-dose of inactivated vaccines and 2-
586 dose of mRNA vaccines and COVID-19 convalescents assayed at 1.3, 6.2 and 12
587 months after infection *(2, 14, 18)*.
588 **(E)** Normalized ELISA binding (EC_{50}) by antibodies isolated from the 3-dose
589 inactivated and 2-dose mRNA vaccinees (ref) as well as COVID-19 convalescents to
590 SARS-CoV-2 S trimer (left) and normalized pseudovirus neutralization activity
591 (IC_{50}) (right) against SARS-CoV-2 assayed at 1.3, 6.2 and 12 months after infection
592 (ref). Among these, eight antibodies reported by Michel's group were expressed and
593 assessed for both binding by ELISA and pseudovirus neutralization activity for
594 normalized comparison here. Black horizontal bars indicate mean values.
595 **(F)** BLI binding affinities (upper panel) and pseudo-typed virus neutralization
596 (bottom panel) by antibodies isolated from the 3-dose vaccinees to circulating SARS-
597 CoV-2 variants. Color gradient for upper panel indicates K_D values ranging from 0
598 (green), through 2.5 (yellow) and 5 (red) to 25 nM (purple). Gray suggests no/very
599 limited binding activity (>1000 nM). Color gradient for bottom panel indicates IC_{50}
600 values ranging from 0 (green), through 20 (yellow) and 200 (red) to 2000 ng/ml
601 (purple). Gray suggests no/very limited neutralizing activity (>2000 ng/ml).
602
603
604
605
606
607
608
609

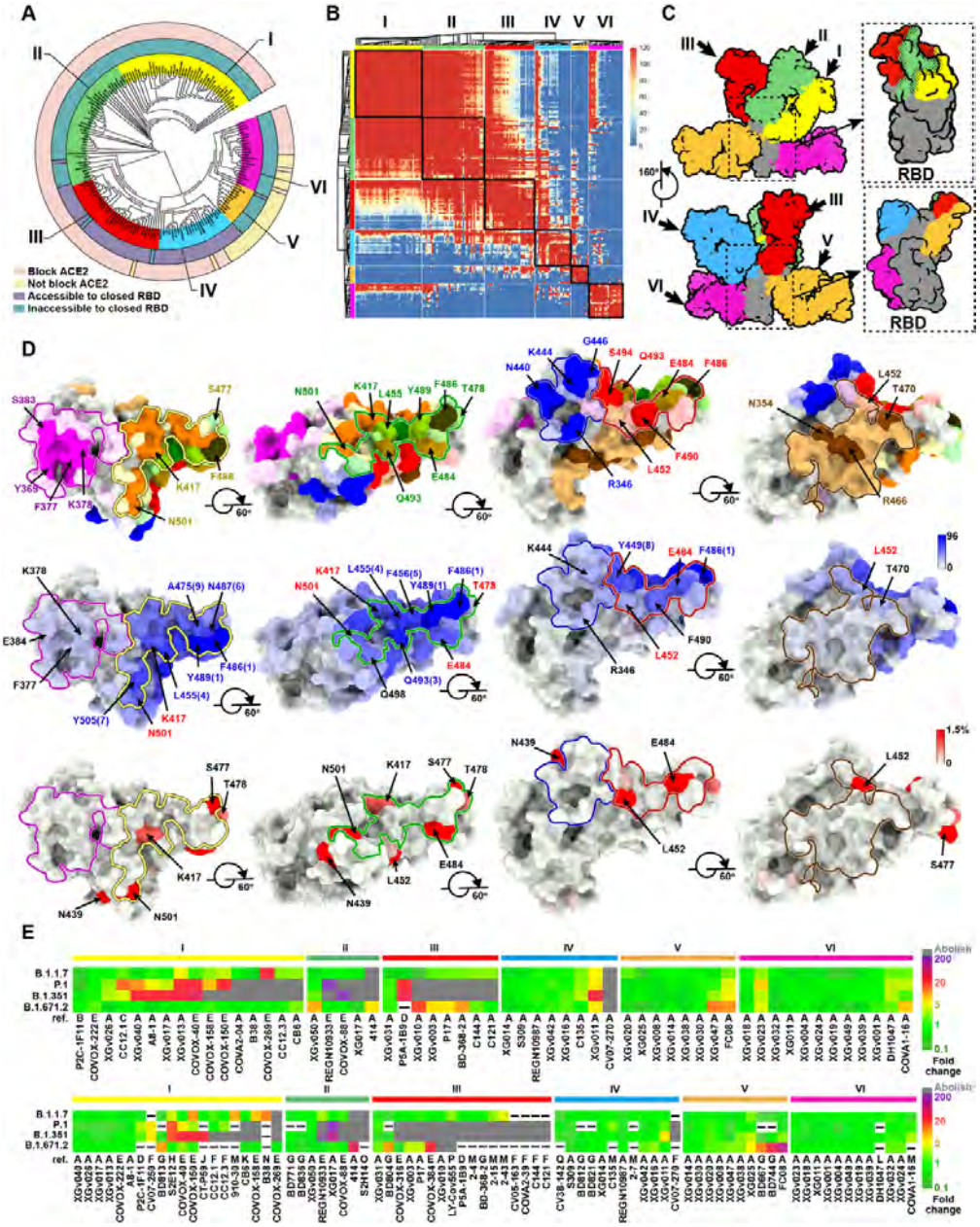


Fig. 3 Structural landscape and immunogenic features of RBD NAbs

(A) Structure-based antigenic clustering of SARS-CoV-2 RBD NAbs. A total of 171 RBD NAbs with available structures were classified into six clusters (I, II, III, IV, V and VI). NAbs that can block ACE2 binding or not are outlined by light pink and light yellow, respectively. NAbs that can attach to the closed RBD or not are outlined by gray blue and gray green, respectively.

(B) Superimposition matrix of 171 RBD NAb structures' output from clashed areas ($Å^2$) between variable regions of any two Fab fragments showing the clustering into

six antibody classes.

(C) Surface representative model of six types of NAbs bound to the RBD. Fab fragments of six representative antibodies are shown in different colors and the RBD is colored in gray. Insets illustrate the antigenic patches targeted by six representative antibodies. Dashed dots indicate the overlaps between two adjacent antigenic patches.

(D) Structural landscapes of the six classes of RBD NAbs (upper panel). Antigenic patches (with targeting frequency >30%) recognized by six classes of NAbs are outlined in the assigned color scheme (same to Fig. 3C), among which residues with "hot targeting frequency" (generally over 65%, but over 85% in class I) are shown in bright colors corresponding to the patches they belong to. Residues involved in two (such as Y489, L452) or three (such as F486) neighboring antigenic patches are presented in a mixed color. Representative "hot" antigenic residues are labeled. Middle: hot map for antigenic residues on the RBD. Per residue frequency recognized by the 171 NAbs were calculated and shown. The top 9 of the hottest antigenic residues and key residues with substitutions in several VOCs are marked and labeled. Bottom: hot map for circulating variants with mutations on the RBD. Mutation frequency for each residue was calculated based on the datasets from GISAID.

(E) Immunogenic characteristics of six classes of RBD-targeting NAbs. Hot maps show relative fold changes in K_D values (up) and IC_{50} values (down) against several VOCs for the six classes of NAbs, including previously reported *(97-108)* and newly isolated antibodies described in this manuscript. Color gradients for upper and bottom panels indicate relative fold changes and are shown at right side. "-": no related datasets in the original studies and related references are listed. Ref "A" indicates that the datasets were produced in this manuscript. Other letters in Ref correspond to different reference numbers shown as below. B – 91 and this manuscipt, C – 99 and this manuscript, D – 97, E – 30, 81, 103 and 104, F – 99, G – 98, H – 100 and 108, J – 101, K – 94 and 102, L – 105 and 106, M – 94, N – 105, O – 107, P – 82, Q – 66, respectively.

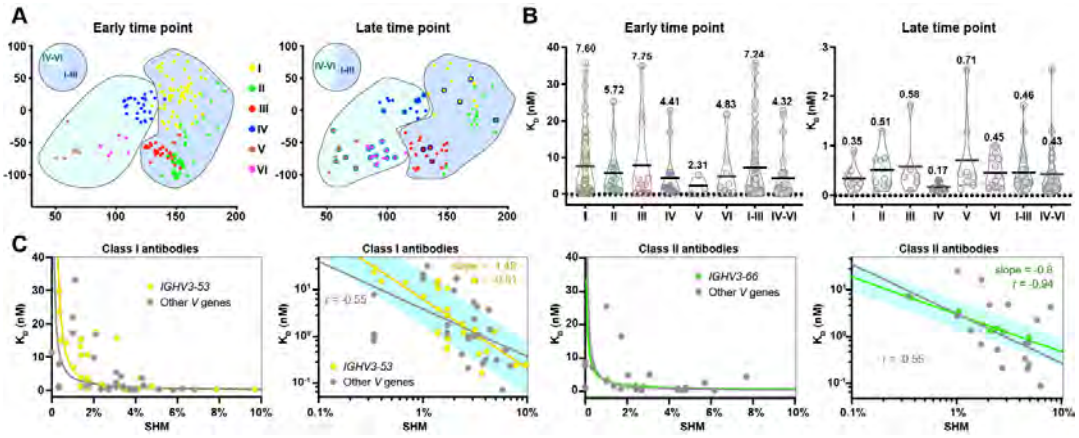


Fig. 4 Antibody evolution and affinity maturation

(A) Uniform manifold approximation and projection (UMAP) plot displaying the antibodies defined as the early time point group (left) and late time point group (right). The antibodies are colored based on their cluster assignments by the hierarchical clustering algorithm. Antibodies from I to III and IV to VI are highlighted in cyan and gray blue background, respectively. Pie charts represent the frequency distribution of antibodies belonging to I to III and IV to VI. Antibodies isolated from 3-dose vaccinees are outlined by black lines.

(B) Dissociation constants (K_D) of the antibodies from I to VI. Individual class antibodies are represented in colors corresponding to the classes they belong to. The color scheme is same as Fig. 4A. BLI traces are shown in fig. S9.

(C) The measured K_D–SHM plots (left) and K_D–SHM log-log plots (right) of antibodies from I and II are shown. *IGHV3-53* and *IGHV3-66* antibodies belonging to class I and II are colored in yellow and green, respectively. The straight curves and lines are the least squares fits of the data to the power law with the values of the slope for *IGHV3-53* and *IGHV3-66* antibodies. The black curves and lines indicate the fitting of antibodies from I or II; the yellow and green ones suggest the fitting of *IGHV3-53* and *IGHV3-66* antibodies, respectively. The cyan lines are the 90% predicted interval.

References and Notes:

1. N. G. Davies, S. Abbott *et al.*, Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. *Science* **372**, (2021); published online EpubApr 9 (10.1126/science.abg3055).
2. Z. Wang, F. Schmidt *et al.*, mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. *nature*, 616–622 (2021)10.1038/s41586-021-03324-6).
3. A. Muik, A. K. Wallisch *et al.*, Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine-elicited human sera. *Science* **371**, 1152-1153 (2021); published online EpubMar 12 (10.1126/science.abg6105).
4. W. F. Garcia-Beltran, E. C. Lam *et al.*, Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell* **184**, 2372-2383 e2379 (2021); published online EpubApr 29 (10.1016/j.cell.2021.03.013).
5. E. Hacisuleyman, C. Hale *et al.*, Vaccine Breakthrough Infections with SARS-CoV-2 Variants. *N Engl J Med* **384**, 2212-2218 (2021); published online EpubJun 10 (10.1056/NEJMoa2105000).
6. E. C. Sabino, L. F. Buss *et al.*, Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. *Lancet* **397**, 452-455 (2021); published online EpubFeb 6 (10.1016/S0140-6736(21)00183-5).
7. W. N. Chia, F. Zhu *et al.*, Dynamics of SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study. *The Lancet. Microbe* **2**, e240-e249 (2021); published online EpubJun (10.1016/S2666-5247(21)00025-2).
8. A. T. Widge, N. G. Rouphael *et al.*, Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination. *N Engl J Med* **384**, 80-82 (2021); published online EpubJan 7 (10.1056/NEJMc2032195).
9. Y. Zhang, G. Zeng *et al.*, Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* **21**, 181-192 (2021); published online EpubFeb (10.1016/S1473-3099(20)30843-4).
10. Q. Gao, L. Bao *et al.*, Development of an inactivated vaccine candidate for SARS-CoV-2. *Science* **369**, 77-81 (2020); published online EpubJul 3 (10.1126/science.abc1932).
11. D. S. Khoury, A. K. Wheatley *et al.*, Measuring immunity to SARS-CoV-2 infection: comparing assays and animal models. *Nature reviews. Immunology* **20**, 727-738 (2020); published online EpubDec (10.1038/s41577-020-00471-1).
12. L. Riepler, A. Rossler *et al.*, Comparison of Four SARS-CoV-2 Neutralization Assays. *Vaccines* **9**, (2020); published online EpubDec 28 (10.3390/vaccines9010013).
13. Q. W. Hongxing Pan, G. Zeng, Immunogenicity and safety of a third dose, and immune persistence of CoronaVac vaccine in healthy adults aged 18-59 years: interim results from a double-blind, randomized, placebo-controlled phase 2 clinical trial. *medRxiv*, (2021)https://doi.org/10.1101/2021.07.23.21261026).
14. Z. Wang, F. Muecksch *et al.*, Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature* **595**, 426-431 (2021); published online EpubJul (10.1038/s41586-021-03696-9).
15. F. Muecksch, Y. Weisblum *et al.*, Development of potency, breadth and resilience to viral escape mutations in SARS-CoV-2 neutralizing antibodies. *bioRxiv*, (2021); published online EpubMar 8 (10.1101/2021.03.07.434227).
16. C. Gaebler, Z. Wang *et al.*, Evolution of antibody immunity to SARS-CoV-2. *Nature* **591**, 639-644 (2021); published online EpubMar (10.1038/s41586-021-03207-w).
17. Y. Zhou, Z. Liu *et al.*, Enhancement versus neutralization by SARS-CoV-2 antibodies from a convalescent donor associates with distinct epitopes on the RBD. *Cell Rep* **34**, 108699 (2021); published online EpubFeb 2 (10.1016/j.celrep.2021.108699).
18. D. F. Robbiani, C. Gaebler *et al.*, Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* **584**, 437-442 (2020); published online EpubAug

(10.1038/s41586-020-2456-9).
19. A. C. Walls, Y. J. Park *et al.*, Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* **181**, 281-292 e286 (2020); published online EpubApr 16 (10.1016/j.cell.2020.02.058).
20. D. Wrapp, N. Wang *et al.*, Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **367**, 1260-1263 (2020); published online EpubMar 13 (10.1126/science.abb2507).
21. R. Yan, R. Wang *et al.*, Structural basis for bivalent binding and inhibition of SARS-CoV-2 infection by human potent neutralizing antibodies. *Cell Res* **31**, 517-525 (2021); published online EpubMay (10.1038/s41422-021-00487-9).
22. W. Dejnirattisai, D. Zhou *et al.*, The antigenic anatomy of SARS-CoV-2 receptor binding domain. *Cell* **184**, 2183-2200 e2122 (2021); published online EpubApr 15 (10.1016/j.cell.2021.02.032).
23. M. Yuan, H. Liu *et al.*, Structural basis of a shared antibody response to SARS-CoV-2. *Science* **369**, 1119-1123 (2020); published online EpubAug 28 (10.1126/science.abd2321).
24. F. Bertoglio, V. Fuhner *et al.*, A SARS-CoV-2 neutralizing antibody selected from COVID-19 patients binds to the ACE2-RBD interface and is tolerant to most known RBD mutations. *Cell Rep* **36**, 109433 (2021); published online EpubJul 27 (10.1016/j.celrep.2021.109433).
25. S. A. Clark, L. E. Clark *et al.*, Molecular basis for a germline-biased neutralizing antibody response to SARS-CoV-2. *bioRxiv*, (2020); published online EpubNov 13 (10.1101/2020.11.13.381533).
26. B. B. Banach, G. Cerutti *et al.*, Paired heavy and light chain signatures contribute to potent SARS-CoV-2 neutralization in public antibody responses. *bioRxiv*, (2021); published online EpubJan 3 (10.1101/2020.12.31.424987).
27. B. E. Jones, P. L. Brown-Augsburger *et al.*, The neutralizing antibody, LY-CoV555, protects against SARS-CoV-2 infection in nonhuman primates. *Sci Transl Med* **13**, (2021); published online EpubMay 12 (10.1126/scitranslmed.abf1906).
28. Y. Guo, L. Huang *et al.*, A SARS-CoV-2 neutralizing antibody with extensive Spike binding coverage and modified for optimal therapeutic outcomes. *Nat Commun* **12**, 2623 (2021); published online EpubMay 11 (10.1038/s41467-021-22926-2).
29. S. Du, Y. L. Cao *et al.*, Structurally Resolved SARS-CoV-2 Antibody Shows High Efficacy in Severely Infected Hamsters and Provides a Potent Cocktail Pairing Strategy. *Cell* **183**, 1013-+ (2020); published online EpubNov 12 (10.1016/j.cell.2020.09.035).
30. W. Dejnirattisai, D. M. Zhou *et al.*, Antibody evasion by the P.1 strain of SARS-CoV-2. *Cell* **184**, 2939-+ (2021); published online EpubMay 27 (10.1016/j.cell.2021.03.055).
31. C. O. Barnes, C. A. Jette *et al.*, SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. *Nature* **588**, 682-+ (2020); published online EpubDec 24 (10.1038/s41586-020-2852-1).
32. N. C. Wu, M. Yuan *et al.*, An Alternative Binding Mode of IGHV3-53 Antibodies to the SARS-CoV-2 Receptor Binding Domain. *Cell Reports* **33**, (2020); published online EpubOct 20 (10.1016/J.Celrep.2020.108274).
33. H. J. Liu, N. C. Wu *et al.*, Cross-Neutralization of a SARS-CoV-2 Antibody to a Functionally Conserved Site Is Mediated by Avidity. *Immunity* **53**, 1272-+ (2020); published online EpubDec 15 (10.1016/j.immuni.2020.10.023).
34. N. K. Hurlburt, E. Seydoux *et al.*, Structural basis for potent neutralization of SARS-CoV-2 and role of antibody affinity maturation. *Nature Communications* **11**, (2020); published online EpubOct 27 (10.1038/S41467-020-19231-9).
35. Y. Wu, F. R. Wang *et al.*, A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. *Science* **368**, 1274-+ (2020); published online EpubJun 12 (10.1126/science.abc2241).

36. R. Shi, C. Shan *et al.*, A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature* **584**, 120-+ (2020); published online EpubAug 6 (10.1038/s41586-020-2381-y).
37. J. W. Ge, R. K. Wang *et al.*, Antibody neutralization of SARS-CoV-2 through ACE2 receptor mimicry. *Nature Communications* **12**, (2021); published online EpubJan 11 (10.1038/s41467-020-20501-9).
38. C. O. Barnes, A. P. West *et al.*, Structures of Human Antibodies Bound to SARS-CoV-2 Spike Reveal Common Epitopes and Recurrent Features of Antibodies. *Cell* **182**, 828-+ (2020); published online EpubAug 20 (10.1016/j.cell.2020.06.025).
39. H. P. Yao, Y. Sun *et al.*, Rational development of a human antibody cocktail that deploys multiple functions to confer Pan-SARS-CoVs protection. *Cell Research* **31**, 25-36 (2021); published online EpubJan (10.1038/s41422-020-00444-y).
40. N. Wang, Y. Sun *et al.*, Structure-based development of human antibody cocktails against SARS-CoV-2. *Cell Research* **31**, 101-103 (2021); published online EpubJan (10.1038/s41422-020-00446-w).
41. S. Miersch, Z. Li, Tetravalent SARS-CoV-2 Neutralizing Antibodies Show Enhanced Potency and Resistance to Escape Mutations. *BioRxiv*, (2021)https://doi.org/10.1101/2020.10.31.362848).
42. C. J. Bracken, S. A. Lim *et al.*, Bi-paratopic and multivalent VH domains block ACE2 binding and neutralize SARS-CoV-2. *Nature chemical biology* **17**, 113-121 (2021); published online EpubJan (10.1038/s41589-020-00679-1).
43. C. Zhang, Y. F. Wang *et al.*, Development and structural basis of a two-MAb cocktail for treating SARS-CoV-2 infections. *Nature Communications* **12**, (2021); published online EpubJan 11 (10.1038/s41467-020-20465-w).
44. L. Piccoli, Y. J. Park *et al.*, Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-+ (2020); published online EpubNov 12 (10.1016/j.cell.2020.09.037).
45. J. Hansen, A. Baum *et al.*, Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010-1014 (2020); published online EpubAug 21 (10.1126/science.abd0827).
46. J. Kreye, S. M. Reincke *et al.*, A Therapeutic Non-self-reactive SARS-CoV-2 Antibody Protects from Lung Pathology in a COVID-19 Hamster Model. *Cell* **183**, 1058-+ (2020); published online EpubNov 12 (10.1016/j.cell.2020.09.049).
47. R. Rouet, O. Mazigi *et al.*, Potent SARS-CoV-2 binding and neutralization through maturation of iconic SARS-CoV-1 antibodies. *Mabs-Austin* **13**, (2021); published online EpubJan 1 (10.1080/19420862.2021.1922134).
48. M. A. Tortorici, M. Beltramello *et al.*, Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* **370**, 950-+ (2020); published online EpubNov 20 (10.1126/science.abe3354).
49. E. Rujas, I. Kucharska *et al.*, Multivalency transforms SARS-CoV-2 antibodies into ultrapotent neutralizers. *Nature Communications* **12**, (2021); published online EpubJun 16 (10.1038/S41467-021-23825-2).
50. J. Ahmad, J. Jiang *et al.*, Synthetic nanobody-SARS-CoV-2 receptor-binding domain structures identify distinct epitopes. *bioRxiv*, (2021); published online EpubJan 27 (10.1101/2021.01.27.428466).
51. M. Schoof, B. Faust *et al.*, An ultrapotent synthetic nanobody neutralizes SARS-CoV-2 by stabilizing inactive Spike. *Science* **370**, 1473-1479 (2020); published online EpubDec 18 (10.1126/science.abe3255).
52. M. Rapp, Y. C. Guo *et al.*, Modular basis for potent SARS-CoV-2 neutralization by a prevalent VH1-2-derived antibody class. *Cell Reports* **35**, (2021); published online EpubApr 6 (10.1016/J.Celrep.2021.108950).

53. C. Kim, D. K. Ryu *et al.*, A therapeutic neutralizing antibody targeting receptor binding domain of SARS-CoV-2 spike protein. *Nature Communications* **12**, (2021); published online EpubJan 12 (10.1038/s41467-020-20602-5).
54. D. Li, R. J. Edwards *et al.*, In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies. *Cell*, (2021); published online EpubJun 18 (10.1016/j.cell.2021.06.021).
55. L. Hanke, L. V. Perez *et al.*, An alpaca nanobody neutralizes SARS-CoV-2 by blocking receptor interaction. *Nature Communications* **11**, (2020); published online EpubSep 4 (10.1038/S41467-020-18174-5).
56. J. D. Huo, A. Le Bas *et al.*, Neutralizing nanobodies bind SARS-CoV-2 spike RBD and block interaction with ACE2 (vol 27, pg 846, 2020). *Nature Structural & Molecular Biology* **28**, 326-326 (2021); published online EpubMar (10.1038/s41594-021-00566-w).
57. B. Ju, Q. Zhang *et al.*, Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature* **584**, 115-+ (2020); published online EpubAug 6 (10.1038/s41586-020-2380-z).
58. Y. L. Cao, B. Su *et al.*, Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-Throughput Single-Cell Sequencing of Convalescent Patients' B Cells. *Cell* **182**, 73-+ (2020); published online EpubJul 9 (10.1016/j.cell.2020.05.025).
59. L. H. Liu, P. F. Wang *et al.*, Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature* **584**, 450-+ (2020); published online EpubAug 20 (10.1038/s41586-020-2571-7).
60. P. A. Koenig, H. Das *et al.*, Structure-guided multivalent nanobodies block SARS-CoV-2 infection and suppress mutational escape. *Science* **371**, 691-+ (2021); published online EpubFeb 12 (10.1126/science.abe6230).
61. Y. F. Xiang, S. Nambulli *et al.*, Versatile and multivalent nanobodies efficiently neutralize SARS-CoV-2. *Science* **370**, 1479-1484 (2020); published online EpubDec 18 (10.1126/science.abe4747).
62. L. Zhu, Y. Q. Deng *et al.*, Double lock of a potent human therapeutic monoclonal antibody against SARS-CoV-2. *National Science Review* **8**, (2021); published online EpubMar (10.1093/nsr/nwaa297).
63. G. Cerutti, M. Rapp *et al.*, Structural basis for accommodation of emerging B.1.351 and B.1.1.7 variants by two potent SARS-CoV-2 neutralizing antibodies. *Structure* **29**, 655-+ (2021); published online EpubJul 1 (10.1016/j.str.2021.05.014).
64. T. F. Custodio, H. Das *et al.*, Selection, biophysical and structural analysis of synthetic nanobodies that effectively neutralize SARS-CoV-2. *Nature Communications* **11**, (2020); published online EpubNov 4 (10.1038/S41467-020-19204-Y).
65. J. F. Scheid, C. O. Barnes *et al.*, B cell genomics behind cross-neutralization of SARS-CoV-2 variants and SARS-CoV. *Cell* **184**, 3205-+ (2021); published online EpubJun 10 (10.1016/j.cell.2021.04.032).
66. H. J. Liu, M. Yuan *et al.*, A combination of cross-neutralizing antibodies synergizes to prevent SARS-CoV-2 and SARS-CoV pseudovirus infection. *Cell Host & Microbe* **29**, 806-+ (2021); published online EpubMay 12 (10.1016/j.chom.2021.04.005).
67. D. Pinto, Y. J. Park *et al.*, Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290-295 (2020); published online EpubJul (10.1038/s41586-020-2349-y).
68. T. N. Starr, N. Czudnochowski *et al.*, Antibodies to the SARS-CoV-2 receptor-binding domain that maximize breadth and resistance to viral escape. *bioRxiv*, (2021).
69. D. M. Zhou, H. M. E. Duyvesteyn *et al.*, Structural basis for the neutralization of SARS-CoV-2 by an antibody from a convalescent patient. *Nature Structural & Molecular Biology* **27**, 950-+ (2020); published online EpubOct (10.1038/s41594-020-0480-y).
70. M. Yuan, N. C. Wu *et al.*, A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. *Science* **368**, 630-+ (2020); published online

EpubMay 8 (10.1126/science.abb7269).
71. Z. Lv, Y. Q. Deng *et al.*, Structural basis for neutralization of SARS-CoV-2 and SARS-CoV by a potent therapeutic antibody. *Science* **369**, 1505-+ (2020); published online EpubSep 18 (10.1126/science.abc5881).
72. Y. L. Cao, A. Yisimayi *et al.*, Humoral immune response to circulating SARS-CoV-2 variants elicited by inactivated and RBD-subunit vaccines. *Cell Research* **31**, 732-741 (2021); published online EpubJul (10.1038/s41422-021-00514-9).
73. Y. Sun, L. Wang *et al.*, Structure-based development of three- and four-antibody cocktails against SARS-CoV-2 via multiple mechanisms. *Cell Research* **31**, 597-600 (2021); published online EpubMay (10.1038/s41422-021-00497-7).
74. X. Zhu, D. Mannar *et al.*, Cryo-electron microscopy structures of the N501Y SARS-CoV-2 spike protein in complex with ACE2 and 2 potent neutralizing antibodies. *PLoS Biol* **19**, e3001237 (2021); published online EpubApr (10.1371/journal.pbio.3001237).
75. D. Asarnow, B. Wang *et al.*, Structural insight into SARS-CoV-2 neutralizing antibodies and modulation of syncytia. *Cell* **184**, 3192-+ (2021); published online EpubJun 10 (10.1016/j.cell.2021.04.033).
76. X. J. Zhou, F. G. Ma *et al.*, Diverse immunoglobulin gene usage and convergent epitope targeting in neutralizing antibody responses to SARS-CoV-2. *Cell Reports* **35**, (2021); published online EpubMay 11 (10.1016/j.celrep.2021.109109).
77. M. F. Jennewein, A. J. MacCamy *et al.*, Isolation and characterization of cross-neutralizing coronavirus antibodies from COVID-19+ subjects. *Cell Rep* **36**, 109353 (2021); published online EpubJul 13 (10.1016/j.celrep.2021.109353).
78. T. N. Starr, N. Czudnochowski *et al.*, SARS-CoV-2 RBD antibodies that maximize breadth and resistance to escape. *Nature*, 1-9 (2021).
79. J. Fedry, D. L. Hurdiss *et al.*, Structural insights into the cross-neutralization of SARS-CoV and SARS-CoV-2 by the human monoclonal antibody 47D11. *Science Advances* **7**, (2021); published online EpubJun (10.1126/sciadv.abf5632).
80. K. Westendorf, S. Zentelis *et al.*, LY-CoV1404 potently neutralizes SARS-CoV-2 variants. *bioRxiv*, (2021); published online EpubMay 4 (10.1101/2021.04.30.442182).
81. C. Liu, H. M. Ginn *et al.*, Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. *Cell*, (2021); published online EpubJun 17 (10.1016/j.cell.2021.06.020).
82. L. Wang, T. Zhou *et al.*, Ultrapotent antibodies against diverse and highly transmissible SARS-CoV-2 variants. *Science*, (2021); published online EpubJul 1 (10.1126/science.abh1766).
83. L. Zhang, L. Cao *et al.*, A proof of concept for neutralizing antibody-guided vaccine design against SARS-CoV-2. *National Science Review*, (2020)10.1093/nsr/nwab053).
84. J. Huo, Y. Zhao *et al.*, Neutralization of SARS-CoV-2 by Destruction of the Prefusion Spike. *Cell Host Microbe* **28**, 445-454 e446 (2020); published online EpubSep 9 (10.1016/j.chom.2020.06.010).
85. M. McCallum, A. De Marco *et al.*, N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *Cell* **184**, 2332-2347 e2316 (2021); published online EpubApr 29 (10.1016/j.cell.2021.03.028).
86. G. Cerutti, Y. Guo *et al.*, Potent SARS-CoV-2 neutralizing antibodies directed against spike N-terminal domain target a single supersite. *Cell Host Microbe* **29**, 819-833 e817 (2021); published online EpubMay 12 (10.1016/j.chom.2021.03.005).
87. X. Y. Chi, R. H. Yan *et al.*, A neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-2. *Science* **369**, 650-+ (2020); published online EpubAug 7 (10.1126/science.abc6952).
88. W. N. Voss, Y. X. J. Hou *et al.*, Prevalent, protective, and convergent IgG recognition of SARS-CoV-2 non-RBD spike epitopes. *Science* **372**, 1108-+ (2021); published online

EpubJun 4 (10.1126/science.abg5268).
89. A. Rosa, V. E. Pye *et al.*, SARS-CoV-2 can recruit a heme metabolite to evade antibody immunity. *Science Advances* **7**, (2021); published online EpubMay (10.1126/sciadv.abg7607).
90. Y. F. Liu, W. T. Soh *et al.*, An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by antibodies. *Cell* **184**, 3452-+ (2021); published online EpubJun 24 (10.1016/j.cell.2021.05.032).
91. P. J. M. Brouwer, T. G. Caniels *et al.*, Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. *Science* **369**, 643-+ (2020); published online EpubAug 7 (10.1126/science.abc5902).
92. T. Noy-Porat, A. Mechaly *et al.*, Therapeutic antibodies, targeting the SARS-CoV-2 spike N-terminal domain, protect lethally infected K18-hACE2 mice. *Iscience* **24**, (2021); published online EpubMay 21 (10.1016/j.isci.2021.102479).
93. J. Goike, C. L. Hsieh *et al.*, Synthetic repertoires derived from convalescent COVID-19 patients enable discovery of SARS-CoV-2 neutralizing antibodies and a novel quaternary binding modality. *bioRxiv*, (2021); published online EpubApr 9 (10.1101/2021.04.07.438849).
94. P. Wang, M. S. Nair *et al.*, Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature* **593**, 130-135 (2021); published online EpubMay (10.1038/s41586-021-03398-2).
95. J. Lopez Bernal, N. Andrews *et al.*, Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. *N Engl J Med*, (2021); published online EpubJul 21 (10.1056/NEJMoa2108891).
96. A. Sheikh, J. McMenamin *et al.*, SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness. *Lancet* **397**, 2461-2462 (2021); published online EpubJun 26 (10.1016/S0140-6736(21)01358-1).
97. R. Wang, Q. Zhang *et al.*, Analysis of SARS-CoV-2 variant mutations reveals neutralization escape mechanisms and the ability to use ACE2 receptors from additional species. *Immunity* **54**, 1611-1621 e1615 (2021); published online EpubJul 13 (10.1016/j.immuni.2021.06.003).
98. P. L. Shuo Du, Zhiying Zhang, Structures of SARS-CoV-2 B.1.351 neutralizing antibodies provide insights into cocktail design against concerning variants. *bioRxiv*, (2021)https://doi.org/10.1101/2021.07.30.454402).
99. M. Yuan, D. Huang *et al.*, Structural and functional ramifications of antigenic drift in recent SARS-CoV-2 variants. *Science*, (2021); published online EpubMay 20 (10.1126/science.abh1139).
100. R. T. E. Chen, X. W. Zhang *et al.*, Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. *Nature Medicine* **27**, (2021); published online EpubApr (10.1038/s41591-021-01294-w).
101. D. K. Ryu, R. Song *et al.*, Therapeutic effect of CT-P59 against SARS-CoV-2 South African variant. *Biochem Biophys Res Commun* **566**, 135-140 (2021); published online EpubAug 20 (10.1016/j.bbrc.2021.06.016).
102. P. Wang, R. G. Casner *et al.*, Increased resistance of SARS-CoV-2 variant P.1 to antibody neutralization. *Cell Host Microbe* **29**, 747-751 e744 (2021); published online EpubMay 12 (10.1016/j.chom.2021.04.007).
103. D. Zhou, W. Dejnirattisai *et al.*, Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. *Cell* **184**, 2348-2361 e2346 (2021); published online EpubApr 29 (10.1016/j.cell.2021.02.037).
104. P. Supasa, D. Zhou *et al.*, Reduced neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera. *Cell* **184**, 2201-2211 e2207 (2021); published online EpubApr 15 (10.1016/j.cell.2021.02.033).
105. X. Y. Shen, H. L. Tang *et al.*, SARS-CoV-2 variant B.1.1.7 is susceptible to neutralizing

antibodies elicited by ancestral spike vaccines. *Cell Host & Microbe* **29**, 529-+ (2021); published online EpubApr 14 (10.1016/j.chom.2021.03.002).

106. D. R. Martinez, A. Schaefer *et al.*, A broadly neutralizing antibody protects against SARS-CoV, pre-emergent bat CoVs, and SARS-CoV-2 variants in mice. *bioRxiv*, (2021); published online EpubApr 28 (10.1101/2021.04.27.441655).

107. D. A. Collier, A. De Marco *et al.*, Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies. *Nature* **593**, 136-141 (2021); published online EpubMay (10.1038/s41586-021-03412-7).

108. R. E. Chen, E. S. Winkler *et al.*, In vivo monoclonal antibody efficacy against SARS-CoV-2 variant strains. *Nature*, (2021); published online EpubJun 21 (10.1038/s41586-021-03720-y).

CoronaVac

O que a ciência comprova

2.9. Países que optaram por vacinas de vírus inativado, como CoronaVac, estão mais protegidos contra variantes do SARS-CoV-2, aponta estudo espanhol

Um estudo realizado por pesquisadores da Universidade de Barcelona, na Espanha, concluiu que vacinas contra a Covid-19 elaboradas com vírus inativado, como é o caso da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, conferem maior eficácia no médio e no longo prazo no controle da pandemia, na comparação com imunizantes feitos com outras tecnologias, devido a seu desempenho diante das variantes do vírus SARS-CoV-2.

Segundo Joan Serrano-Marín e Rafael Franco, autores do artigo "Two urgent needs in the battle against COVID-19: a classic-type vaccine and specific medication", publicado na plataforma de preprints OSF, as novas tecnologias de vacinas desenvolvidas em ritmo emergencial para o combate à pandemia, como RNA mensageiro e vetor viral de adenovírus, podem conferir proteção elevada frente à cepa original do SARS-CoV-2, mas tendem a perder eficácia à medida que vão emergindo novas variantes.

"As vacinas clássicas, como a CoronaVac, promovem a geração de um repertório mais amplo de anticorpos e respostas celulares. Ou seja, elas nos permitem neutralizar o vírus seguindo estratégias mais diversas. Prova disso é a situação positiva que vivem países como Chile, China e Uruguai, onde a principal vacina utilizada tem sido a CoronaVac", explicam Joan e Rafael em entrevista exclusiva para o Portal do Butantan.

Os imunizantes de vírus inativado contêm todas as partes do vírus morto. Isso pode gerar uma resposta imune mais abrangente que as das vacinas de RNA mensageiro ou que usam adenovírus como vetor viral, já que elas utilizam somente uma parte da proteína Spike (utilizada pelo SARS-CoV-2 para infectar as células).

O artigo sugere que a reinfecção e o colapso dos sistemas de saúde podem ocorrer em países que usam as vacinas de RNA mensageiro ou de adenovírus, embora a porcentagem da população vacinada seja alta – assim como aconteceu em Israel. A mesma tendência, ou seja, novas ondas pandêmicas após a vacinação em massa com vacinas de RNA/adenovírus, estaria sendo vista, de acordo com os pesquisadores, em vários países europeus e nos Estados Unidos.

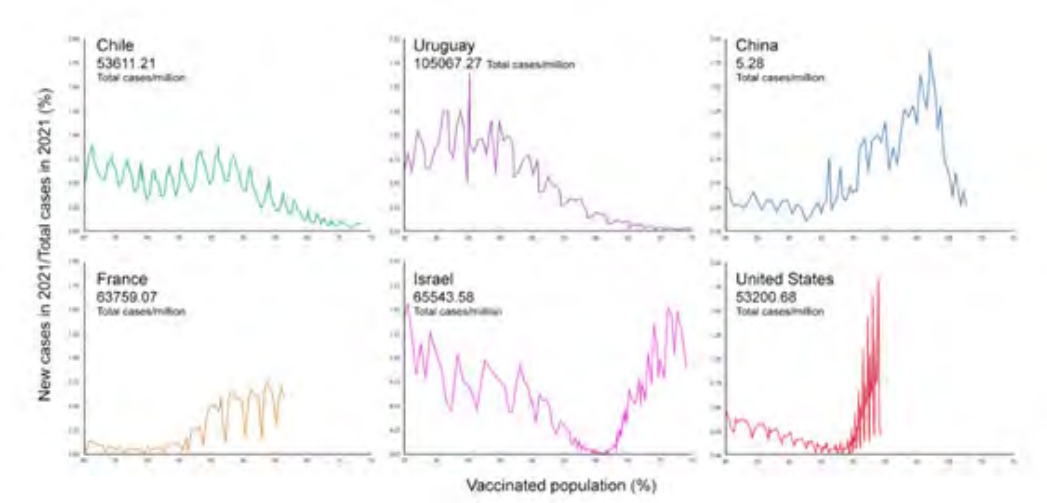
"A carga viral da variante delta é muito alta para vacinados e não vacinados. Em outras palavras, os vacinados continuarão infectando os vacinados e os não vacinados. A imunidade de rebanho, em termos gerais, é alcançada quando o número médio de infectados infecta menos de uma pessoa por infectado. Ou seja, é preciso reduzir drasticamente a transmissão. Conforme indicam os cálculos realizados, para o mesmo percentual de vacinados, a transmissão é extremamente menor nos países que utilizaram a CoronaVac como vacina principal", complementam Joan e Rafael.

Desempenho das vacinas de vírus inativado

Países como Estados Unidos, Israel e Reino Unido têm enfrentado um recrudescimento no número de casos de Covid-19, apesar dos altos índices de vacinação. O motivo é a chegada da variante delta (B.1.617.2, indiana), mais transmissível. É uma tendência oposta ao que se observa no Chile, Uruguai e China, que usaram a CoronaVac como principal imunizante.

Nos casos do Uruguai e do Chile, o aumento da porcentagem de vacinação da população com CoronaVac levou a uma redução considerável na proporção de novos casos. Em relação à China, os cientistas ressalvam que nem os aumentos nem as quedas são significativos, pois o total de 2.021 casos, medido por milhão de habitantes, é insignificante na comparação com os outros países (cinco novos casos por milhão de habitantes na China, contra 65.543 em Israel ou 53.200 nos Estados Unidos).

Para os pesquisadores, a administração da CoronaVac e outros imunizantes de vírus inativado é altamente desejável para a obtenção da imunidade coletiva devido ao amplo espectro de anticorpos que elas geram nos indivíduos vacinados, incluindo uma maior diversidade e quantidade de anticorpos neutralizantes e não neutralizantes, e sua maior capacidade de responder às possíveis mutações ou deriva genética de todas as proteínas do SARS-CoV-2.



"O maior número de estratégias imunológicas que as vacinas tradicionais induzem se deve principalmente ao fato de que, partindo do vírus completo, no caso da CoronaVac, o sistema imunológico é capaz de induzir um maior repertório de respostas, tornando esse processo mais eficaz. Isto não acontece com as vacinas modernas, de RNA mensageiro ou de adenovírus, todas elas concebidas para focar sua ação em única proteína do coronavírus, a proteína S, que também pode sofrer mutação quando o vírus sofre mutação", resumem Joan e Rafael.

Como funcionam as vacinas de vírus inativado

Cada dose de vacina de vírus inativado, cuja tecnologia é conhecida há mais de um século, é composta por trilhões de partículas do vírus em questão. Por serem inativadas, tais partículas são incapazes de provocar a doença em quem recebe o imunizante. Sua função é outra: estimular o sistema imune a reconhecer o vírus assim que entrar em contato com ele.

Como a CoronaVac contém o vírus SARS-CoV-2 inteiro inativado, o sistema imune produz anticorpos que reconhecem muitos antígenos (proteínas) do novo coronavírus. A proteína S é a principal delas, usada pelo SARS-CoV-2 para penetrar nas células humanas, mas não a única. O coronavírus conta ao todo com 29 proteínas, em sua grande maioria responsáveis por regular a multiplicação e a saída do vírus das células humanas. Sendo assim, uma variante que apresenta alteração da proteína S (mutação) deixa de ser reconhecida por vacinas específicas contendo somente a proteína S.

As vacinas modernas foram concebidas de modo a conferir ao sistema imune a habilidade de identificar a proteína S, estimulando assim a produção de anticorpos neutralizantes, que são as principais armas do nosso organismo no combate ao vírus. Já vacinas de modelo tradicional, como a CoronaVac, por conter o vírus inteiro, são capazes de estimular o sistema imune a reconhecer em maior ou menor grau todas as proteínas, disparando a produção tanto de anticorpos neutralizantes da proteína S, quanto de diversos outros relacionados às demais proteínas do arsenal viral.

Publicado em: 31/08/2021

Two urgent needs in the battle against COVID-19: a classic-type vaccine and specific medication

Joan Serrano-Marín [1] and Rafael Franco [1,2,3,*]

[1] Dept. Biochemistry and Molecular Biomedicine. University of Barcelona, 08028 Barcelona. Spain.
[2] School of Chemistry. University of Barcelona, 08028 Barcelona. Spain.
[3] Network Center: Neurodegenerative diseases (CiberNed). Spanish National Health Institute Carlos III. 28034 Madrid. Spain.

* Correspondence: Rafael Franco, Dept. Biochemistry and Molecular Biomedicine. University of Barcelona. Diagonal 643. Prevosti Building. 08028 Barcelona. Catalonia. Spain; rfranco@ub.edu; rfranco1234@gmail.com; Tel.: +34-4021208 (R.F.)

Abstract: The COVID-19 pandemic has led to the development of vaccines against the causative virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The need for urgent release of anti-SARS-CoV-2 tools has motivated the approval of a new vaccines never used before for mass vaccination, some based on RNA (mRNA vaccines) and some using an adenoviral vector (AV vaccines). Despite high nominal efficacy, in some populations the actual numbers seem to be lower due to several factors that include new viral variants that scape from the immunological response elicited by the vaccines, which have led to new pandemic waves. In fact, the proportion of new cases has decreased in Countries using a classic-type vaccine (inactivated), CoronaVac. In the current August 2021 scenario there is a need to prevent infection, transmission and to diminish the symptoms of the disease by drug repurposing and/or development of ad hoc medication. This manuscript has two aims. On the one hand, it highlights the need to develop classic-type vaccines and to approve them in the US and in Europe. Without classic-type vaccines, herd immunity is unlikely to be achieved. On the other hand, the paper comments on different therapeutic approaches to reduce the severity of COVID-19 and the number of deaths.

Keywords: Vaccine booster, CoronaVac; Sputnik V; adenovirus; RNA vaccines; renin-angiotensin system; viral proteases.

Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been the worst pandemic since the so-called Spanish flu in 1918. The number of deaths and affected people around the world, in only two years, is incredibly high and the return to normal life is not expected anytime soon. As of today (August 10, 2021; https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---10-August-2021) the number of affected people is estimated to be >150 million and >3.5 million deaths, often with >10,000 occurring in a single day.

There is no approved drug/intervention to specifically fight the virus once a person is infected. Antibodies extracted from recovered or convalescent individuals may be useful (1–3), although there are doubts about their general efficacy and/or the correct protocol for use (4). Therefore, the first line of defense to stop pandemics is mass vaccination. The success in the fight against the coronavirus is based, mainly, on the speed with which the different vaccines have been developed, approved and produced. Vaccines aim to develop immunological mechanisms to stop infection, disease transmission and/or the worst consequences of infection. This is accomplished by challenging the immunological system with antigens made up of viral proteins. In the fight against SARS-CoV-2, the most successful option has been to combine new-technology vaccines including part of the nucleotide sequence coding for the spike protein. This makes sense, as the

spike is the protein that interacts with the main SARS-CoV-2 receptor on the target cell, namely angiotensin converting enzyme 2 (ACE2).

The production of the spike protein to be directly used in a vaccine is not an easy task. In fact, the spike S proteins of coronaviruses contain from 1104 to 1273 amino acids (5). Rapidly producing the huge amounts needed for the worldwide vaccination of hundreds, even thousands, of millions of people is a challenge that was never undertaken. An alternative option is to make the vaccine with a nucleic acid that encodes for the protein (in whole or in part). While it is difficult to produce and purify the protein *in vitro*, thus keeping its natural conformation and antigenicity, it is more feasible to produce the nucleic acids that encode for the protein. This approach has therefore been adopted with success in terms of efficacy against infection and production speed. Two types of nucleic acids have been used: RNA and DNA. In mRNA vaccines, the coding sequence is in the form of messenger RNA (mRNA), which enters the cells of vaccinated individuals and can be easily converted into the spike protein. To deliver the mRNA to the cells, a lipid-based encapsulation/nanoparticle can be used. In DNA vaccines, the DNA coding sequence for the spike protein can be delivered with viral vectors, like for instance those based on adenovirus (AV), which is a non-enveloped DNA virus. AVs were being developed as vaccines for diseases such as Ebola (6), but the COVID-19 pandemic has shifted the focus to the production and approval for emergency use of AV vaccines against SARS-CoV-2.

In terms of current vaccines using sequences coding for the spike protein and being administered worldwide, Pfizer and Moderna vaccines are based on RNA, whereas AstraZeneca, Johnson & Johnson and Sputnik V vaccines are based on AV, i.e. on DNA. At present (August 10) the ones approved in the European Union are those from Pfizer, Moderna, AstraZeneca, and Johnson & Johnson. In the United States, all except the AstraZeneca vaccine have obtained emergency use authorization. In other countries the vaccine developed in Russia, Sputnik V, is being tested with supposedly high efficacy rates and there are still doubts on its approval in the European Union. In China and some countries in South America, a classic type vaccine is the one that is mainly used. Looking at the whole picture one does not understand why in the EU and in the US no classic-type vaccine has been developed and approved by regulatory bodies. For decades classic-type vaccines have been developed using methods that have been successful in fighting a variety of diseases (7,8). Since the pioneering work of Louis Pasteur developing a vaccine against the rabies virus (See (9)), they have proven effective in the prevention of serious diseases caused by viruses (see WHO global vaccine Action plan: https://www.who.int/teams/immunization-vaccines-and-biologicals/strategies/global-vaccine-action-plan; accessed on August 16, 2021).

Benefits versus risks associated to new vaccines

First and foremost, the new mRNA and AV vaccines developed to fight COVID-19 are generally safe, at least in the short-term. However, due to the urgency to stop spreading SARS-CoV-2, they have been approved in less than one year after the outbreak of the SARS-CoV-2 pandemic. For one thing, possible long-term problems of vaccinated people due to a specific vaccine have not been empirically addressed. Even though, considering the preexistent bibliography, these effects are very unlikely to happen, this issue cannot be ignored considering the huge number of people receiving these vaccines. On the other hand, urgency has prevented the appearance of classic vaccines, which have shown in the past an impeccable efficacy and safety record (10,11). Accordingly, although mRNA/AV vaccines may be instrumental to achieving large numbers of short-term vaccinated people around the world, classic-type vaccines must also be considered. By August 2021, there are two classic-type vaccines approved for human use; both have been developed in China: Covilo or BBIBP-CorV (from Sinopharm) and CoronaVac (from Sinovac Research and Development) (https://www.who.int/es/news-room/q-a-detail/coronavirus-disease-(covid-19)-vaccines; accessed on August 16, 2021).

Despite the obvious benefits of reducing infections and deaths in vaccinated people, the risks must be brought to the table. The risks of thrombi for humans receiving the AstraZeneca or Johnson & Johnson vaccines are serious, but can be weighed against the risk-benefit assessment. Due to the high number of variables, it is difficult to reliably compare the percentage of cases with thrombus versus the total number of vaccinations with the overall risk of death in unvaccinated people. But it is reasonable to accept that the relatively low number of cases with thrombosis should not stop vaccination with AstraZeneca or Johnson & Johnson vaccines. However, caution should be exercised when these vaccines are administered to people taking medications in which one of the potential side effects is thrombus formation; the most obvious case is certain types of birth control pills. Another risk of the mRNA/AV vaccines is the possibility of integration of exogenous material into the DNA of host cells (12). AVs have been tested for decades as vectors in gene therapy and the problems of their use have led to the development of safer vectors such as adeno-associated viruses (see (13) for review).

The risk is seemingly lower in the case of mRNA vaccines, but it has been demonstrated that genetic material of SARS-CoV-2 can be converted into DNA that integrates into the human genome (12,14). The human genome does not include the gene for any typical reverse transcriptase, but it includes retrotransposons that can "move" using a copy and paste mechanism that requires a RNA intermediate. Accordingly, retrotransposon may act as instruments to convert RNA from viruses or mRNA vaccines into genomic DNA (12,14). One of the deciphered mechanisms is mediated by the LINE-1 retrotransposable element ORF2 protein (15,16). The human genome contains several full or truncated sequences of long interspersed element-1 retrotransposons and it is assumed that >80 of those elements can be transcribed; random integration of elements in the genome has been related to a variety of diseases (15,17,18). Interestingly, SARS-CoV-2 infection alters the usual dynamics of some transposable elements, such as LINEs, increasing their expression and, therefore, the probability of insertion of new transposable elements (19). Additionally, SARS-CoV-2 is not the only RNA virus with positive polarity (that is, that is directly transcribed by the host cell ribosomes) that has the capability of directly interacting retrotransposons; among others, Hepatitis C (16) or Sindbis (20) viruses may interact with transposons. In summary, the integration of exogenous genetic material into host genome may lead to risks, such as premature cell death or tumor cell growth, that cannot be addressed in the short term, i.e. before emergence use anti-COVID-19 vaccine approval.

The efficacy issue

The efficacy of a vaccine is not a direct measure of its capacity to avoid the symptoms of the COVID-19. In the case of the vaccines, efficacy cannot be measured as in the case of a drug for a disease, from diabetes to Alzheimer's. Efficacy of antidiabetic medication is measured in patients that take the drug and after some period of time the reduction in plasma glucose levels are measured. Few clinical parameters are needed, just the glycemia and the percentage of reduction that is considered as end point. If a 20% is selected, the efficacy is measured by the number of patients whose levels are reduced by more than 20% versus the total number of patients. In Alzheimer's disease the end point consists of increasing the score in a cognition test, for instance the mini-mental test (MMSE: Mini-Mental State Examination). The main parameters needed to test any anti-dementia medication are to select the range of scores of patients to recruit and to select the minimum expected score increase in the MMSE scale.

More parameters plus some ad hoc assumptions are needed for efficacy assessment of vaccines. First and foremost, vaccinated people does not have any disease. Then, it is not possible to assess efficacy by directly looking at whether or not vaccinated people have been cured or have fewer symptoms of the disease. The first assumption is that vaccinated individuals will have similar exposure to SARS-CoV-2 than non-vaccinated individuals (or placebo inoculated individuals). Fortunately, an ad hoc surrogate marker for vaccine efficacy is the level of IgGs in plasma, mainly

of neutralizing antibodies, i.e. antibodies that prevent infection. Unfortunately, in SARS-CoV-2 it is important to know the level of the IgGs but also the composition of IgGs. The serological quick tests have demonstrated that different COVID-19-suffering individuals produce different antibodies. In other words, quick tests, which nominally have >90% sensitivity, lead to false negatives, i.e. sensitivity may be >90% in one given infected population and may be far lower in another infected population. Plasma from convalescent patients show a mixture of anti-SARS-CoV-2 antibodies (21). Microfluidic devices have shown that humoral responses to coronavirus can elicit with a variety of antigen / antibody interaction affinities (22). To make thinks even more complicated, many of the vaccination schedules include two shots and this adds complexity to the estimation of the real preventive effect of anti-COVID vaccines. Taken together, it is almost impossible to estimate the efficacy of any vaccine with reliability. In addition, the neutralizing antibodies, i.e. those that impede infection, are unknown and/or may be neutralizing for a given strain of the virus but not for a different one. In practical terms, only the big pharma has the potential to enroll thousand individuals and to provide an efficacy estimates to apply for approval by regulatory bodies. Also, the efficacy data may vary from trial to trial, and or by adding more data if the trial is extended. It has been common for the companies developing the mRNA/AV vaccines to present, upon time, increases in the percentage of efficacy for the same vaccine. The poor efficacy values of classic-type is surely behind the decision to stop the development of some vaccines such as the TMV-083 (previously known as MV-SARS-CoV-2), which was developed by one of the most experienced institutes in the World, the Pasteur Institute (23) (see https://www.pasteur.fr/en/all-sars-cov-2-covid-19-institut-pasteur/research-projects/covid-19-vaccine-against-sars-cov-2-infection-using-measles-vector; accessed on April 19, 2021) and its partner company: Sanofi.

In summary, mRNA/AV vaccines have prevented deaths, but they have not been able to stop the spread of the virus and have favored the appearance of new variants. It is essential to have vaccines that not only prevent death, but also stop transmission and genetic shift/drift. In addition a very recent paper reporting clinical research with individuals vaccinated with RNA vaccines states: "*we document significant declines in antibody levels three months post-vaccination, and reduced neutralization of emerging variants*" (24).

The third dose issue

The use of vaccines that are not able to stop the transmission has contributed to selection of viruses with mutated forms of the spike protein. This issue was, among others, raised by Nobel Laureate Luc Montagnier. He doubted that vaccination to stop COVID-19 spread was convenient due to the appearance of new variants. No doubt vaccination has been instrumental to decrease the death toll, but novel SARS-CoV-2 variants have arisen that are able to lead to COVID-19 symptoms in vaccinated people. The current pandemic is due to a virus with a high transmission capacity, which means that a given individual may be exposed to the virus more than once and in relatively short periods of time. It is often forgotten that all people, vaccinated or not, may be infected by any SARS-CoV-2 variant. But mRNA/AV vaccines that use the sequence (DNA or RNA) of a given spike protein, may not be efficacious in attenuation infection/symptoms produced by new variants. In fact, more and more vaccinated people are being re-infected and able to infect close contacts. For instance, the AstraZeneca vaccine (ChAdOx1 nCoV-19) has shown a highly reduced efficacy, among others, against the B.1.351 variant. In summary, mRNA/AV vaccines have been useful but have led to new variants in a selection-escape fashion. In the search for convincing data to obtain vaccine approval, clinical trials with two injections were designed (with the exception of the Janssen vaccine). On the one hand, two shots surely lead to a higher production of anti-spike antibodies in serum and this may be convincing for regulatory bodies. On the other hand, two shots may be needed and/or convenient for viruses that do not have high mutation rates. However, two shots to combat a virus RNA that mutates

so rapidly is, quite likely, not the best option. Worse, here are chances of approval of a third shot of the same vaccine. Taken together, all available information and basic knowledge of the human immune system, indicates that a third dose with the same vaccine is not the best option. Fortunately, there is an alternative that, importantly, has already proven with high success, namely the use of a classic-type vaccine. By previous knowledge with this type of vaccines, the selection of new variants would be minimal and, in addition, "classical" vaccines lead to more efficient immunological tools, humoral and cellular, to fight SARS-CoV-2 via diverse components and not only via the spike protein.

Vaccination that allows viral escape by mutation will compromise the control of pandemics and the achievement of herd immunity. In reality, countries that are using mRNA/AV vaccines anticipate that herd immunity will not be achieved in such a scenario, complementary approaches should be sought (25). To combat the escape of the human immunodeficiency virus (HIV) by mutation, the so-called Highly Active Antiretroviral (HAART) or "triple" therapy was developed for acquired immunodeficiency syndrome (AIDS) patients. While one single drug was not efficacious to control the disease, the combination of three different compounds prevented mutations thus allowing disease control. The triple therapy consisted of inhibitors of two relevant components of HIV-1, the reverse transcriptase and the main viral protease (26,27). AIDS is now considered a chronic disease that produces few direct deaths. Currently, it is not possible to prevent the escape of SARS-CoV-2 by mutation using drugs, but the availability of different types of vaccines opens a window of opportunity. In the same way that a single drug is not effective for AIDS patients, a single vaccine can reduce the number of deaths, but it can allow a viral escape by mutation, a reduction in the effectiveness of the vaccine and an inability to achieve herd immunity. Accordingly, more shots of the very same vaccine will have a limited benefit in comparison with shots of a heterologous vaccine (28,29). More shots of the same vaccine may be detrimental on putting pressure to the virus thus selecting more infective viral particles. Recent developments in the anti-HIV-1 research field include the use of combining vaccines that, to combat the HIV-1 pandemic "*must induce responses capable of controlling vast HIV-1 variants circulating in the population as well as those evolved in each individual following transmission*" (30). In summary, despite the lack of a drug cocktail, a combination of different vaccines is emerging as a real alternative to effectively combat SARS-CoV-2. Obviously, the optimal treatment would not be to use vaccines directed against the same protein, that is, the SARS-CoV-2 spike protein. In European countries and in the US, all vaccines are directed against the spike protein. Should these countries approve vaccines of a different type (non-RNA-based, non AV-based) and/or directed against other viral components?

New cases after 30% population vaccination using new- or classic-type vaccines

Available data suggests that reinfection and collapse of emergency units at hospitals may occur in countries using the mRNA/AV vaccines even though the percentage of vaccinated population is high (31,32). Perhaps the main example is Israel that was among the quickest in vaccinating with mRNA/AV vaccines. The same trend, i.e. new waves after massive vaccination with mRNA/AV vaccines, has occurred in various European Countries and in the US. This trend is opposite in the only three countries that used the CoronaVac vaccine as the main vaccine (Figure 1).

Figure 1 shows the trend of new cases in three Countries mainly using CoronaVac and in three Countries using mRNA/AV vaccines. Despite alarms in Uruguay, it is clear that increasing the percentage of population vaccination with CoronaVac has led to a dramatic decrease in the proportion of new cases. Something similar has occurred in another Country mainly using CoronaVac, Chile. The data available for China suggests an increase followed by a sharp decrease, but it should be noted that neither the rises nor the falls are significant as total 2021 cases, measured per 1,000,000 inhabitants, are negligible in China compared to the other selected countries (5 in China versus 65,543 in Israel or 53,200 in the US, date: August

25). In sharp contrast, France, Israel and the US shows an increase of new cases upon increased vaccination using mRNA/AV vaccines.

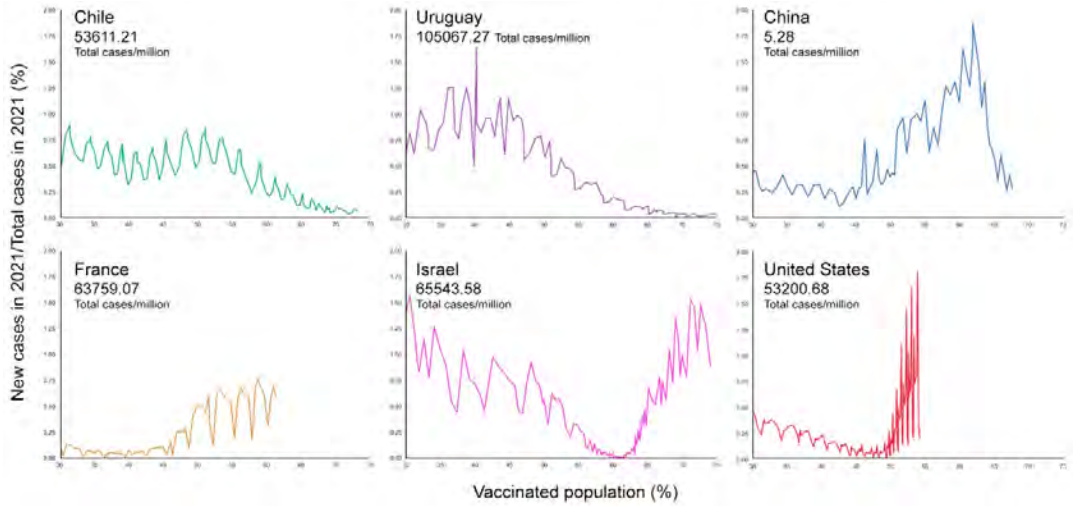


Figure 1. New COVID-19 cases versus percentage of vaccinated population. Data (retrieved until August 24, 2021) have been selected using 30% vaccinated population as threshold. Chile, Uruguay and China have mainly used CoronaVac vaccine. France, Israel and the US have used only mRNA/AV vaccines. The numbers below the name of the Country indicate total reported cases from the beginning of 2021. For comparison purposes the same axis, X and Y, were used in all graphics. A file with the data used construct the graphics, coming from repositories containing official data reported by the Countries (see "Data availability statement" below).

For statistical analysis we have considered 10 countries (US, Israel, Greece, Portugal, Ireland, Italy, Spain, United Kingdom, Denmark and France) that have not used CoronaVac but mRNA/AV vaccines, and the only three countries using CoronaVac as the main vaccine (>70% administrated doses at date August 24, 2021), China, Chile and Uruguay. Data were retrieved from a big data source, Github (https://github.com/owid/covid-19-data/tree/master/public/data), which is forged with COVID-19-related data in official webs such as in the Oxford COVID-19 Government Response Tracker or in independent global health research centers such as the Institute for Health Metrics and Evaluation at the University of Washington. The Excel file containing all data was directly downloaded from Github (https://covid.ourworldindata.org/data/owid-covid-data.xlsx; accessed (on August 24, 2021; see "Data availability statement" below). The interaction graphic was obtained using Statgraphics v. 18.1.14 from a general linear model analysis with type of vaccine (mRNA/AV or CoronaVac) as a qualitative factor, % vaccinated population as a quantitative factor and, as a dependent variable, the relative % positives in 2021 (which is the relation of new positives after reaching 30% of the vaccinated population and the total positives in 2021. The 30% threshold was set up because a lower percentage of vaccination has little effect on pandemic indicators). Although vaccination begun at the end of 2020 and the beginning of 2021, only data from 2021 were analyzed. To avoid interference due to differential public health decisions and differences in the timing and rate of vaccination in each country, no attempt was made to make comparisons between countries using similar vaccines. We have found a very significant correlation between the percentage of population receiving the mRNA/AV vaccination (full regime; two shots except for the Johnson & Johnson vaccine, which is administered in only one shot) and number of new cases after reaching 30% vaccination of the population in a given Country, namely cases in 2021 after reaching 30% vaccination versus total cases in 2021. The two lines (one for mRNA/AV viruses and another for CoronaVac) are of opposite slope, i.e. correlations are opposite when considering CoronaVac or the vaccines based in mRNA/AV. Whereas the ratio of cases after 30% vaccination increases with further vaccination with mRNA/AV vaccines, the ratio decreases in countries where CoronaVac

is used. In fact, statistical analysis shows significance for a differential trend using CoronaVac or mRNA/AV vaccines. The correlation was done using proportion of cases as quantitative variable and type of vaccine as qualitative variable. The significance holds if only three countries using the mRNA/AV vaccines are considered, i.e. considering data from 3 countries in both sets of data. The significance also holds taking out the data from China, whose management of the pandemic has been quite different to that in many other countries.

In summary, vaccination with mRNA/AV vaccines does not stop transmission, while in countries that use the CoronaVac vaccine, cases decrease with increasing population vaccination rate, suggesting effective neutralization that may eventually lead to herd immunity.

The need of a classic-type vaccine

A complete schedule of a mRNA/AV vaccine, two doses of Pfizer, Moderna or AstraZeneca, and one dose of the Johnson & Johnson vaccine, as many organizations define including The Pan American Health Organization/ World Health Organization (https://ais.paho.org/imm/IM_DosisAdmin-Vacunacion.asp), in 50% of the population has not eradicated the virus and, worse, new waves of infections have appeared. In our Country (Spain) we were, at the end of July 2021, in the mid of the fifth wave and there are officials stating (August 20) that the sixth wave is coming. In elderly houses in Catalonia (Spain) in which all residents are vaccinated (>90% with mRNA vaccines) there is a surge of new cases (August 2021; official data in: https://dadescovid.cat/?drop_es_residencia=1). This was not expected when vaccination started. Some of the reasons of having such unexpected scenario may be now figured out.

On the one hand, and apart from the reduction upon time of the antibody levels *(see above;* **The efficacy issue** section), it is known that significant amounts of mucosal IgA is associated with less viral transmission. Likewise, in all the viral infections studied to date, a higher proportion of IgA at the epithelial level reduces the risk of re-infection (33). Therefore, the production of IgAs is important to reduce (upon vaccination) re-infection and associated transmissibility (34). Not all vaccines have confirmed production of IgAs at the mucosal level; a recent publication reports IgAs secretion to human milk after shots of Pfizer's vaccine (35). This finding is important for preventing infection of the neonate, but the relevance in epidemiological terms is under question. Efficacious prevention of the infection requires production of aggregated, secretory, forms of IgA (SIgA), whose affinity for antigens is much higher than monomeric IgA (36). Therefore, one indicator of the effectiveness of a vaccine is the number of mucosal SIgAs and whether they are neutralizing or not (36). The few studies on this matter suggest that IgA production by mRNA/AV vaccines is, at the very least, very modest (37), and this seems to be one of the reason of low efficacy in reducing infection and transmission despite the high nominal values of efficacy in producing antibodies (33).

On the other hand, although it is commonly thought that the only antibodies capable of preventing infection are neutralizing antibodies, non-neutralizing antibodies are important irrespective of their later involvement in the viral replication cycle (38). In this sense, classic-type vaccines lead, by definition, to a more qualitative diverse repertoire of neutralizing and non-neutralizing antibodies than vaccines only based in producing IgG against a single viral protein.

The CoronaVac vaccine, developed by a Chinese company, Sinovac Research and Development, consists of inactivated SARS-CoV-2 and aluminum hydroxide as adjuvant. It has been among the first vaccines to be developed and at present is being tested in different countries (39). Only in China 1 million people was already vaccinated by the end of 2020 in a phase III clinical trial that started in November 2020. Fewer data about CoronaVac are available in English if compared with the huge amount of information available (in English) for the other vaccines. Although a direct comparison between classic-type vaccine and mRNA/AV vaccines is difficult to perform, some

reviews on this theme have recently appeared (see, for instance (39–41)). A recent paper compares data from 13 clinical trials of 11 different vaccines, taken both reports in English and in Chinese. The conclusion of the authors is that: "*Most of the COVID-19 vaccines appear to be effective and safe. Double-dose vaccination is recommended. However, more research is needed to investigate the long-term efficacy and safety of the vaccines and the influence of dose, age, and production process on the protective efficacy*" (42).

It is remarkable and far from being generally known by the population and by Western Health authorities that, CoronaVac lacks the serious side effects identified for RNA- and AV vaccines (43), namely, clot formation, Guillain-Barré syndrome, myocarditis, etc. Additionally, vaccine developers already have experience on controlling pandemics with inactivated vaccines, such as that caused by the poliovirus at the beginning of the 20th century, whose mutation rate is similar to that of SARS-CoV-2 (44,45) and whose basic reproduction number (R_0) throughout the pandemic was not different from that of the coronavirus (46,47). In summary, mRNA/AV vaccines have instrumental for the quickness in being approved and for the high nominal efficacy rate but classic-type vaccines are needed and the only one already developed shows that it should enter into the vaccination program to combat COVID-19 in all over the World.

Safety, tolerability and immunogenicity was successfully addressed in a first phase I/II trial in volunteers of the Suining County of Chinese Jiangsu province. One of the outputs of the study was the selection of 3 μg CoronaVac dose for phase III trials, which have been performed in different countries. Approval has been granted already in, among others, China (48), Brazil (https://www.reuters.com/article/us-health-coronavirus-brazil-coronavac-idUSKBN29R2GL; accessed April 23, 2021), Uruguay and Chile (https://www.ispch.cl/noticia/isp-autorizo-la-vacuna-coronavac-del-laboratorio-sinovac-life-sciences-co-ltd-para-uso-de-emergencia-en-el-pais/; accessed April 23, 2021).

Chile, which is a country of reference in anti-COVID-19 vaccination, is using the CoronaVac and the Pfizer vaccines in a 80:20 approximate proportion (80 CoronaVac, 20 Pfizer); the two vaccines are scheduled to be given as two injections. CoronaVac was approved in Chile after the results of a phase III clinical trials performed in the Country. It has been noticed that the efficacy in preventing productive infection, especially after the first shot is modest and comparable to that whose development was stopped by Pasteur/Sanofi, i.e. in the 50-60% range. Remarkably, this low level of efficacy does not result in poor performance and this has been proved by data obtained upon continuing vaccination schedules. The good COVID-19 data in Chile, which is due to the Pfizer and CoronaVac vaccines, strongly suggest that efficacy estimates are not enough to rule out a vaccine. There is strong evidence showing that despite low efficacy estimates, CoronaVac is achieving the key objective, which is to save human lives. Another phase III trial (PROFISCOV Study) was conducted between July 21 and December 16, 2020 in Brazil among healthcare professionals (49,50). The conclusion as posted in Elsevier's SSRN database is that the vaccine was "*efficacious against any symptomatic SARS-CoV-2 infections and highly protective against moderate and severe COVID-19*" (50).

Some of the advantages of vaccines that protect from infection despite having low nominal efficacy values and lower antibody titers than those elicited by mRNA/AV vaccines, may come for an appropriate engagement of T cell responses. The likelihood of requiring robust T helper cell responses to prevent COVID-19 infection has been suggested from a mouse study using recombinant spike proteins (51). In fact, based on previous experience with coronavirus, the risk of antibody-dependent potentiation (ADE) for anti-SARS-CoV-2 is significant, pointing to the need to develop vaccines that are less dependent on antibody production and more than T cell responses (52). In summary, both humoral and cellular responses are needed for an effective fight against this specific coronavirus. Surprisingly, there is evidence of negligible impact of SARS-CoV-2 variants on T-cell responses, i.e. variants that escape the action of antibodies are likely unable to cope with $CD4^+$ and $CD8^+$ T cell reactivity (53). In this sense, CoronaVac apart from

being safe and producing neutralizing antibodies against the receptor binding domain of the S1 spike protein, immunization induced the activation of T cells (when exposed to SARS-CoV-2 antigens) and the secretion of IFN-γ (54). A recent publication shows that one dose of CoronaVac is already effective against the spreading of the P-1 Brazilian variant of the virus (55).

The need of a specific anti-COVID-19 medication

Drugs used at the beginning of the pandemic, including antibiotics and human immunodeficiency virus protease inhibitors, were not at all effective. When noting that the most serious symptom derived from an imbalance in the immune response with exacerbation of the production of pro-inflammatory cytokines that aggravated the pneumonia, the treatment of choice consisted of glucocorticoids. Since vaccines have not been able to fully prevent infection and disease transmission, there is an urgent need to develop specific anti-COVID-19 drugs.

One interesting possibility is to target the renin-angiotensin system (RAS). The rationale is mainly based in the main SARS-CoV-2 receptor, angiotensin converting enzyme 2 (ACE2). This RAS member interacts with other RAS members such as angiotensin II receptors, which belong to the family of G protein-coupled receptors (GPCRs). GPCRs are very druggable and, in fact, are the target of about 40% of approved drugs worldwide. In addition, antagonists of angiotensin receptors are approved to combat hypertension. Accordingly, it would be informative to perform clinical research correlating the RAS status in with disease severity in COVID-19 patients. Parameters to consider are arterial blood pressure values, the use or not of anti-hypertensives and the type of anti-hypertensives, i.e. whether antihypertensives targeting RAS leads to a differential course of the disease compared with using other type of antihypertensives. In addition, targeting RAS members may lead to decrease in infection because RNA viruses need GPCRs to enter into cells and several RAS members are GPCRs and ACE2 interacts with some of those RAS GPCRs (see (56) and references therein). Often, the serious effects of SARS-CoV-2 infection that can eventually lead to death are due to an imbalance of the immune system in which macrophages play a key role (57). A hot topic in the immune system field is to find drugs able to produce M2 macrophages that, opposite to the M1 or proinflammatory macrophages, facilitate the resolution of inflammation. Accordingly, the discovery of targets to produce M2 macrophages is a promising approach to fight against COVID-19.

Soon after the beginning of the pandemics, a laboratory that has been for years involved in coronavirus research solved the structure of the main protease of SARS-CoV-2 (M^{pro} also known as $3CL^{pro}$) also designing specific inhibitors of the alpha-ketoamide type (58). These inhibitors are at the forefront of being used as specific anti-COVID-19 tools (59).

All over the world there are screening of several compound libraries to try to find inhibitors of viral infection. At present several target candidates have been proposed to manage SARS-CoV-2 infection but further research is needed to find the most promising ones in terms of druggability, efficacy and safety (60–62).

Data Availability Statement: Data used to build Figure 1 will be available upon request when the paper becomes published (data retrieved from repositories with official data on COVID-19 from all Countries).

Author Contributions: R.F. participated in conceptualization and wrote the first draft. J.S.M. did the search of the data and performed statistical analysis. Both authors have contributed to prepare the final version and approve submission.

Funding: Not applicable.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: Not applicable

Conflicts of Interest: The authors declare no conflict of interest.

Bibliography

1. Altuntas F, Ata N, Yigenoglu TN, Bascı S, Dal MS, Korkmaz S, et al. Convalescent plasma therapy in patients with COVID-19. Transfus Apher Sci. 2021 Feb 1;60(1):102955.
2. Brener ZZ, Brenner A. Effectiveness of convalescent plasma therapy in a patient with severe COVID-19-associated acute kidney injury. Clin Nephrol Case Stud [Internet]. 2021 Jan 1 [cited 2021 Aug 7];9(1):67. Available from: /pmc/articles/PMC8170122/
3. Budhiraja S, Dewan A, Aggarwal R, Singh O, Juneja D, Pathak S, et al. Effectiveness of convalescent plasma in Indian patients with COVID-19. Blood Cells, Mol Dis. 2021 May 1;88:102548.
4. Piechotta V, Iannizzi C, Chai KL, Valk SJ, Kimber C, Dorando E, et al. Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a living systematic review. Cochrane Database Syst Rev. 2021 May 20;2021(5).
5. Liu Z, Xiao X, Wei X, Li J, Yang J, Tan H, et al. Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. J Med Virol [Internet]. 2020 Jun 1 [cited 2021 Apr 19];92(6):595–601. Available from: https://pubmed.ncbi.nlm.nih.gov/32100877/
6. Tapia MD, Sow SO, Ndiaye BP, Mbaye KD, Thiongane A, Ndour CT, et al. Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in adults in Africa: a randomised, observer-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis [Internet]. 2020 Jun 1 [cited 2021 Aug 16];20(6):707–18. Available from: http://www.thelancet.com/article/S1473309920300165/fulltext
7. Thompson KM, Kalkowska DA. Review of poliovirus modeling performed from 2000 to 2019 to support global polio eradication. https://doi.org/101080/1476058420201791093 [Internet]. 2020 Jul 2 [cited 2021 Aug 17];19(7):661–86. Available from: https://www.tandfonline.com/doi/abs/10.1080/14760584.2020.1791093
8. Willis ED, Woodward M, Brown E, Popmihajlov Z, Saddier P, Annunziato PW, et al. Herpes zoster vaccine live: A 10 year review of post-marketing safety experience. Vaccine. 2017 Dec 19;35(52):7231–9.
9. Hoenig LJ, Jackson AC, Dickinson GM. The early use of Pasteur's rabies vaccine in the United States. Vaccine. 2018 Jul 16;36(30):4578–81.
10. Zuber PL, Autran B, Asturias EJ, Evans S, Hartigan-Go K, Hussey G, et al. Global safety of vaccines: Strengthening systems for monitoring, management and the role of GACVS. Expert Rev Vaccines [Internet]. 2009 [cited 2021 Aug 16];8(6):705–16. Available from: https://www.tandfonline.com/doi/abs/10.1586/erv.09.40
11. Stassijns J, Bollaerts K, Baay M, Verstraeten T. A systematic review and meta-analysis on the safety of newly adjuvanted vaccines among children. Vaccine. 2016 Feb 3;34(6):714–22.
12. Doerfler W. Adenoviral Vector DNA- and SARS-CoV-2 mRNA-Based Covid-19 Vaccines:

Possible Integration into the Human Genome - Are Adenoviral Genes Expressed in Vector-based Vaccines? Virus Res. 2021 Sep;302:198466.

13. Cathomen T. AAV vectors for gene correction. Curr Opin Mol Ther [Internet]. 2004;6(4):360–6. Available from: https://pubmed.ncbi.nlm.nih.gov/15468594/
14. Zhang L, Richards A, Inmaculada Barrasa M, Hughes SH, Young RA, Jaenisch R. Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues.
15. Bao W, Jurka J. Origin and evolution of LINE-1 derived "half-L1" retrotransposons (HAL1). Gene. 2010 Oct;465(1–2):9.
16. Honda T. Links between Human LINE-1 Retrotransposons and Hepatitis Virus-Related Hepatocellular Carcinoma. Front Chem. 2016;0(MAY):21.
17. Goodier JL, Kazazian HH. Retrotransposons Revisited: The Restraint and Rehabilitation of Parasites. Cell. 2008 Oct;135(1):23–35.
18. Kramerov DA, Vassetzky NS. Origin and evolution of SINEs in eukaryotic genomes. Hered 2011 1076. 2011 Jun;107(6):487–95.
19. Yin Y, Liu X, He X, Zhou L. Exogenous Coronavirus Interacts With Endogenous Retrotransposon in Human Cells. Front Cell Infect Microbiol. 2021 Feb;0:55.
20. Roy M, Viginier B, Saint-Michel É, Arnaud F, Ratinier M, Fablet M. Viral infection impacts transposable element transcript amounts in Drosophila. Proc Natl Acad Sci. 2020 Jun;117(22):12249–57.
21. Hansen J, Baum A, Pascal KE, Russo V, Giordano S, Wloga E, et al. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. Science (80-) [Internet]. 2020 Aug 21 [cited 2021 Apr 22];369(6506):1010–4. Available from: https://pubmed.ncbi.nlm.nih.gov/32540901/
22. Fiedler S, Piziorska MA, Denninger V, Morgunov AS, Ilsley A, Malik AY, et al. Antibody Affinity Governs the Inhibition of SARS-CoV-2 Spike/ACE2 Binding in Patient Serum. ACS Infect Dis [Internet]. 2021 Apr 20 [cited 2021 Apr 22]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/33876632
23. Karpiński TM, Ożarowski M, Seremak-Mrozikiewicz A, Wolski H, Wlodkowic D. The 2020 race towards SARS-CoV-2 specific vaccines [Internet]. Vol. 11, Theranostics. Ivyspring International Publisher; 2021 [cited 2021 Apr 19]. p. 1690–702. Available from: /pmc/articles/PMC7778607/
24. McDade TW, Demonbreun AR, Sancilio A, Mustanski B, D'Aquila RT, McNally EM. Durability of antibody response to vaccination and surrogate neutralization of emerging variants based on SARS-CoV-2 exposure history. Sci Reports 2021 111 [Internet]. 2021 Aug 30 [cited 2021 Aug 31];11(1):1–6. Available from: https://www.nature.com/articles/s41598-021-96879-3
25. Khateeb J, Li Y, Zhang H. Emerging SARS-CoV-2 variants of concern and potential intervention approaches. Crit Care 2021 251 [Internet]. 2021 Jul 12 [cited 2021 Aug 17];25(1):1–8. Available from: https://ccforum.biomedcentral.com/articles/10.1186/s13054-021-03662-x
26. Van Heeswijk RPG, Veldkamp AI, Mulder JW, Meenhorst PL, Lange JMA, Beijnen JH, et al. Combination of protease inhibitors for the treatment of HIV-1-infected patients: A review of pharmacokinetics and clinical experience. Antivir Ther [Internet]. 2001 [cited 2021 Aug 17];6(4):201–29. Available from: https://pubmed.ncbi.nlm.nih.gov/11878403/
27. Burch LS, Smith CJ, Phillips AN, Johnson MA, Lampe FC. Socioeconomic status and response to antiretroviral therapy in high-income countries: A literature review. AIDS. 2016 May 15;30(8):1147–61.
28. Lu S. Heterologous prime-boost vaccination. Curr Opin Immunol [Internet]. 2009 Jun [cited 2021 Aug 31];21(3):346–51. Available from: https://pubmed.ncbi.nlm.nih.gov/19500964/

29. Zhang J, He Q, An C, Mao Q, Gao F, Bian L, et al. Boosting with heterologous vaccines effectively improves protective immune responses of the inactivated SARS-CoV-2 vaccine. Emerg Microbes Infect [Internet]. 2021 Jan 1 [cited 2021 Aug 31];10(1):1598–608. Available from: https://pubmed.ncbi.nlm.nih.gov/34278956/
30. Wee EG, Moyo N, Hannoun Z, Giorgi EE, Korber B, Hanke T. Effect of epitope variant co-delivery on the depth of CD8 T cell responses induced by HIV-1 conserved mosaic vaccines. Mol Ther - Methods Clin Dev [Internet]. 2021 Jun 11 [cited 2021 Aug 17];21:741–53. Available from: http://www.cell.com/article/S232905012100084X/fulltext
31. Kadri SS, Simpson SQ. Potential Implications of SARS-CoV-2 Delta Variant Surges for Rural Areas and Hospitals. JAMA. 2021 Aug;
32. Twohig KA, Nyberg T, Zaidi A, Thelwall S, Sinnathamby MA, Aliabadi S, et al. Hospital admission and emergency care attendance risk for SARS-CoV-2 delta (B.1.617.2) compared with alpha (B.1.1.7) variants of concern: a cohort study. Lancet Infect Dis. 2021 Aug;0(0).
33. Russell MW, Moldoveanu Z, Ogra PL, Mestecky J. Mucosal Immunity in COVID-19: A Neglected but Critical Aspect of SARS-CoV-2 Infection. Front Immunol. 2020 Nov;0:3221.
34. Sterlin D, Mathian A, Miyara M, Mohr A, Anna F, Claër L, et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. Sci Transl Med. 2021 Jan;13(577):2223.
35. Perl SH, Uzan-Yulzari A, Klainer H, Asiskovich L, Youngster M, Rinott E, et al. SARS-CoV-2–Specific Antibodies in Breast Milk After COVID-19 Vaccination of Breastfeeding Women. JAMA. 2021 May;325(19):2013–4.
36. Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Viant C, Gaebler C, et al. Enhanced SARS-CoV-2 neutralization by dimeric IgA. Sci Transl Med. 2021 Jan;13(577):1555.
37. Wisnewski A V., Luna JC, Redlich CA. Human IgG and IgA responses to COVID-19 mRNA vaccines. PLoS One. 2021 Jun;16(6):e0249499.
38. Mayr L, Su B, Moog C. Role of nonneutralizing antibodies in vaccines and/or HIV infected individuals. Curr Opin HIV AIDS. 2017 May;12(3):209–15.
39. Prüβ BM. Current state of the first covid-19 vaccines. Vaccines [Internet]. 2021 Jan 8 [cited 2021 Apr 19];9(1):1–12. Available from: https://www.mdpi.com/2076-393X/9/1/30
40. Almeida SLAC, Oliveira DC de, Faria LLF de, Godoy MCS, Oliveira MMC de, Loch MAL, et al. Uma análise crítica das vacinas disponíveis para Sars-cov-2 / A critical analysis of the vaccines available for Sars-cov-2. Brazilian J Heal Rev [Internet]. 2021 Mar 4 [cited 2021 Apr 22];4(2):4537–55. Available from: https://www.brazilianjournals.com/index.php/BJHR/article/view/25669
41. Doroftei B, Ciobica A, Ilie O-D, Maftei R, Ilea C. Mini-Review Discussing the Reliability and Efficiency of COVID-19 Vaccines. Diagnostics [Internet]. 2021 Mar 24 [cited 2021 Apr 22];11(4):579. Available from: https://pubmed.ncbi.nlm.nih.gov/33804914/
42. Xing K, Tu X-Y, Liu M, Liang Z-W, Chen J-N, Li J-J, et al. Efficacy and safety of COVID-19 vaccines: a systematic review. Zhongguo Dang Dai Er Ke Za Zhi [Internet]. 2021 Mar [cited 2021 Apr 10];23(3):221–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/33691913
43. Tanriover MD, Doğanay HL, Akova M, Güner HR, Azap A, Akhan S, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. Lancet (London, England). 2021 Jul;398(10296):213.
44. Egeren D Van, Novokhodko A, Stoddard M, Tran U, Zetter B, Rogers M, et al. Risk of rapid evolutionary escape from biomedical interventions targeting SARS-CoV-2 spike protein. PLoS One. 2021;16(4):e0250780.
45. Jorba J, Campagnoli R, De L, Kew O. Calibration of Multiple Poliovirus Molecular Clocks Covering an Extended Evolutionary Range. J Virol. 2008 May;82(9):4429.
46. Anderson R, May R. Directly transmitted infections diseases: control by vaccination.

Science (80-). 1982 Feb;215(4536):1053–60.
47. Fine PEM. Herd Immunity: History, Theory, Practice. Epidemiol Rev. 1993 Jan;15(2):265–302.
48. Lancet Commission on COVID-19 Vaccines and Therapeutics Task Force Members. Operation Warp Speed: implications for global vaccine security. Lancet Glob Heal [Internet]. 2021 Mar 26 [cited 2021 Apr 10]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/33780663
49. Palacios R, Patiño EG, de Oliveira Piorelli R, Conde MTRP, Batista AP, Zeng G, et al. Double-Blind, Randomized, Placebo-Controlled Phase III Clinical Trial to Evaluate the Efficacy and Safety of treating Healthcare Professionals with the Adsorbed COVID-19 (Inactivated) Vaccine Manufactured by Sinovac – PROFISCOV: A structured summary of a study protocol for a randomised controlled trial [Internet]. Vol. 21, Trials. BioMed Central Ltd; 2020 [cited 2021 Apr 10]. Available from: https://pubmed.ncbi.nlm.nih.gov/33059771/
50. Palacios R, Batista AP, Albuquerque CSN, Patiño EG, Santos J do P, Tilli Reis Pessoa Conde M, et al. Efficacy and Safety of a COVID-19 Inactivated Vaccine in Healthcare Professionals in Brazil: The PROFISCOV Study. SSRN Electron J [Internet]. 2021 Apr 13 [cited 2021 Apr 22]; Available from: https://papers.ssrn.com/abstract=3822780
51. Su QD, Zou YN, Yi Y, Shen LP, Ye XZ, Zhang Y, et al. Recombinant SARS-CoV-2 RBD with a built in T helper epitope induces strong neutralization antibody response. Vaccine [Internet]. 2021 Feb 22 [cited 2021 Apr 10];39(8):1241–7. Available from: https://pubmed.ncbi.nlm.nih.gov/33516600/
52. Ricke DO. Two Different Antibody-Dependent Enhancement (ADE) Risks for SARS-CoV-2 Antibodies. Front Immunol [Internet]. 2021 Feb 24 [cited 2021 Apr 10];12. Available from: https://pubmed.ncbi.nlm.nih.gov/33717193/
53. Tarke A, Sidney J, Methot N, Yu ED, Zhang Y, Dan JM, et al. Impact of SARS-CoV-2 variants on the total CD4+ and CD8+ T cell reactivity in infected or vaccinated individuals. Cell Reports Med. 2021 Jul;2(7):100355.
54. Zhang H, Deng S, Ren L, Zheng P, Hu X, Jin T, et al. Profiling CD8+ T Cell Epitopes of COVID-19 Convalescents Reveals Reduced Cellular Immune Responses to SARS-CoV-2 Variants. Cell Rep. 2021 Aug;109708.
55. Ranzani OT, Hitchings MDT, Dorion M, Lang D'agostini T, Cardoso De Paula R, Ferreira Pereira De Paula O, et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study. BMJ. 2021;374:2015.
56. Franco R, Rivas-Santisteban R, Serrano-Marín J, Rodríguez-Pérez AI, Labandeira-García JL, Navarro G. SARS-CoV-2 as a Factor to Disbalance the Renin–Angiotensin System: A Suspect in the Case of Exacerbated IL-6 Production. J Immunol [Internet]. 2020 [cited 2020 Aug 19];205(5):1198–206. Available from: https://pubmed.ncbi.nlm.nih.gov/32680957/
57. Grant RA, Morales-Nebreda L, Markov NS, Swaminathan S, Querrey M, Guzman ER, et al. Circuits between infected macrophages and T cells in SARS-CoV-2 pneumonia. Nature [Internet]. 2021 Jan 11 [cited 2021 Aug 17];590(7847):635–41. Available from: https://www.nature.com/articles/s41586-020-03148-w
58. Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, et al. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science (80-). 2020;368:409-412.
59. Mengist HM, Dilnessa T, Jin T. Structural Basis of Potential Inhibitors Targeting SARS-CoV-2 Main Protease. Front Chem. 2021 Mar 12;0:7.
60. Faheem, Kumar BK, Sekhar KVGC, Kunjiappan S, Jamalis J, Balaña-Fouce R, et al. Druggable targets of SARS-CoV-2 and treatment opportunities for COVID-19. Bioorg Chem [Internet]. 2020;104(September):104269. Available from:

https://doi.org/10.1016/j.bioorg.2020.104269

61. Farombi EO, Adedara IA, Ajayi BO, Ayepola OR, Egbeme EE. Kolaviron, a Natural Antioxidant and Anti-Inflammatory Phytochemical Prevents Dextran Sulphate Sodium-Induced Colitis in Rats. Basic Clin Pharmacol Toxicol [Internet]. 2013 Jul [cited 2019 Jul 21];113(1):49–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23336970

62. Zhou H, Fang Y, Xu T, Ni WJ, Shen AZ, Meng XM. Potential therapeutic targets and promising drugs for combating SARS-CoV-2. Br J Pharmacol [Internet]. 2020 Jul 1 [cited 2021 Aug 16];177(14):3147–61. Available from: https://bpspubs.onlinelibrary.wiley.com/doi/full/10.1111/bph.15092

CoronaVac

O que a ciência comprova

3. É segura para gestantes e para os bebês

3.1. CoronaVac tem eficácia de 85% na prevenção de casos graves de Covid-19 em grávidas, mostra pesquisa

Uma pesquisa realizada por cientistas brasileiros e britânicos mostrou que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, teve eficácia de 85% para evitar casos graves de Covid-19 entre gestantes brasileiras. O estudo foi publicado na plataforma de preprints SSRN, vinculada à revista The Lancet, e seus autores são da London School of Hygiene and Tropical Medicine, da Universidade Federal da Bahia, da Fundação Oswaldo Cruz, da Universidade de Brasília e da Universidade do Estado do Rio de Janeiro.

Segundo os pesquisadores, a eficácia do esquema completo de imunização com duas doses da CoronaVac foi de 85% para evitar casos graves de Covid-19, e de 75% na prevenção da progressão dos casos sintomáticos para a forma grave da doença. Nenhuma morte ocorreu entre as gestantes parcialmente ou totalmente imunizadas com a CoronaVac, enquanto quatro óbitos seriam esperados se a mortalidade fosse a mesma do público não vacinado.

A população estudada foi a de todas as gestantes com sintomas de Covid-19, entre 18 e 49 anos, com registro de teste PCR realizado entre 15/3 e 3/10 de 2021, e registradas no Sistema de Notificação do Ministério da Saúde (e-SUS Notifica). Ao final da triagem, foram selecionados os dados de 19.838 gestantes, sendo que 7.424 (37,4%) haviam testado positivo para Covid-19, e 588 (7,9%) desenvolveram a forma grave da doença. No momento da extração dos dados, 83% das gestantes haviam recebido as duas doses da vacina, enquanto 17% haviam recebido apenas uma dose.

"Um regime completo de CoronaVac em gestantes foi eficaz na prevenção dos casos sintomáticos de Covid-19 e altamente eficaz na prevenção da forma grave da doença", salientaram os pesquisadores.

Em 17/1 de 2021, o Ministério da Saúde iniciou a vacinação contra a Covid-19 com CoronaVac. Em 15/3, mulheres grávidas com comorbidades e em ocupações consideradas de alto risco tornaram-se elegíveis para receber a vacina. Em 26/4, a recomendação da imunização foi expandida para incluir todas as gestantes.

Publicado em: 12/11/2021

Effectiveness of the CoronaVac vaccine in prevention of symptomatic and progression to severe Covid-19 in pregnant women in Brazil

Enny S. Paixao,[1,2*], Kerry LM Wong[1], Flavia Jôse Oliveira Alves[2], Vinicius de Araújo Oliveira[2,3,4] Thiago Cerqueira-Silva[3,4], Juracy Bertoldo Júnior[2,4] Tales Mota Machado[5], Elzo Pereira Pinto Junior[2], Viviane S Boaventura[4], Gerson O. Penna[6], Guilherme Loureiro Werneck[7,8], Laura C. Rodrigues[12], Neil Pearce[1], Mauricio L. Barreto [2,4], Manoel Barral-Netto[2,3,4]

1. London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK
2. Center of Data and Knowledge Integration for Health (CIDACS), Gonçalo Moniz Institute, Oswaldo Cruz Foundation , Salvador, Bahia, Brazil;
3. LIB and LEITV Laboratories, Instituto Gonçalo Moniz, Fiocruz, Salvador, Bahia, Brazil
4. Federal University of Bahia, Salvador, Bahia, Brazil;
5. Universidade Federal de Ouro Preto, Ouro Preto, Brazil;
6. Tropical Medicine Centre, University of Brasília, Fiocruz School of Government Brasília, Brazil (G O Penna PhD).
7. Instituto de Medicina Social, Universidade do Estado do Rio de Janeiro
8. Instituto de Estudos em Saúde Coletiva, Universidade Federal do Rio de Janeiro.

Abstract

Background
The effectiveness of Covid-19 inactivated vaccines in pregnant women is unknown. We estimated vaccine effectiveness (VE) of CoronaVac against symptomatic and severe Covid-19 and in preventing progression from symptomatic to severe Covid-19 in pregnant women in Brazil.

Methods
We conducted a test-negative design study in all pregnant women aged 18 to 49 years in Brazil, linking records of negative and positive SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) tests to national vaccination records. We also linked records of test positive cases with notification of severe, hospitalized or fatal Covid-19. Using logistic regression, we estimated adjusted odds and VE against symptomatic Covid-19 by comparing vaccine status in test positive (confirmed cases) to that in subjects with a negative test result. We also calculated the odds/VE against progression by comparing vaccine status in symptomatic cases to that in severe Covid-19 cases.

Findings
Of 19838 tested pregnant women, 7424 (37.4%) tested positive for Covid-19 and 588 (7.9%) had severe disease. Only 83% of pregnant women who received a first dose of CoronaVac completed the vaccination scheme. A single dose of the CoronaVac vaccine was not effective at preventing symptomatic Covid-19. Effectiveness of two doses of CoronaVac was 41% (95% CI 27.1- 52.2) against symptomatic Covid-19, 85% (95% CI 59.5-94.8) against severe Covid-19 and (75%; 95% CI 27.9- 91.2) in preventing progression to severe Covid-19 among those infected.

Interpretation
A complete regimen of CoronaVac in pregnant women was effective in preventing symptomatic Covid-19, and highly effective against severe illness in a setting that combines high disease burden and elevated Covid-19 related maternal deaths.

Research in Context

Evidence before this study

We searched PubMed for articles published "pregnant women" AND "vaccine" AND "SARS-CoV-2" AND “CoronaVac” AND “effectiveness” no results were found. Additionally, we repeated the search using "pregnant women" AND "vaccine" AND "SARS-CoV-2" AND “effectiveness”. Although pregnant women are at elevated risk of Covid-19 complications, they were excluded from most Covid-19 vaccine trials. The observational studies of vaccine effectiveness (VE) recently conducted were restricted to mRNA vaccines.

Added value of this study

This study observed that a single dose of the CoronaVac vaccine offered no protection against symptomatic Covid-19; a complete regimen of CoronaVac was 41% effective in preventing symptomatic Covid-19, and 85% effective in preventing severe Covid-19 disease; it was 75% effective in preventing severe outcomes in those who had been infected.

Implications of all the available evidence

A complete regimen of CoronaVac in pregnant women was effective in preventing symptomatic Covid-19, and highly effective against severe illness in a setting that combines high disease burden and elevated Covid-19 related maternal deaths.

Introduction

Cardiopulmonary and immune changes during pregnancy induce shifts in immune responses, increasing pregnant women's susceptibility to some infectious-related adverse outcomes.[1] Although pregnant women have higher a risk of Covid-19 complications, need intensive care and mechanical ventilation more often, and have higher fatality,[2] they were excluded from most Covid-19 vaccine trials.[3] There is considerable interest on establishing the safety and efficacy/effectiveness of Covid-19 vaccines in this population.[4] A number of observational studies of vaccine effectiveness (VE) were recently conducted[5,6,7,8], but those studying pregnant women were restricted to mRNA vaccines.[9,10,11,12,13]

Many low- and middle-income countries are conducting vaccination campaigns using CoronaVac,[5] an inactivated-virus vaccine; some countries, like Brazil, offer CoronaVac to pregnant women. On January 17, 2021, the Brazilian Ministry of Health initiated Covid-19 vaccination with two CoronaVac doses with two to four weeks interval between doses. The policy followed internationally agreed priorities.[14] On March 15, 2021, pregnant women with co-morbidities and in occupations considered, on balance, to be at high risk, became eligible to receive Covid-19 vaccine.[15] On April 26, this recommendation was expanded to include all pregnant women.[16] Although the exact figures for pregnant women are unclear, we anticipated that enough pregnant women would have been vaccinated to make it possible to evaluate vaccine effectiveness in pregnant women: Brazil combines a sufficient vaccine coverage (more than 50% of the population with two doses),[17] more than 21 million cases and 600,000 deaths (October 2021),[18] and a considerable number of maternal deaths.[19,20]

In this observational study of routine data in Brazil we estimated the VE of CoronaVac vaccine against symptomatic Covid-19 and in preventing progression from symptomatic to severe Covid-19 disease in pregnant women.

Methods

Objectives and study design

The primary objective of this study was to estimate VE of CoronaVac vaccine against symptomatic cases of Covid-19 in a test negative design (TND) in all pregnant women who had a RT-PCR test. We also estimated the effectiveness of vaccine the against developing severe Covid-19 (comparing severe, hospitalized or fatal Covid-19 with test negatives). As a further consistency check, we estimated VE against progression from symptomatic Covid-19 disease to severe Covid-19 (severe, hospitalized or fatal) by comparing the vaccine status of

those who developed severe disease with those who tested positive but did not develop severe disease.

Data sources

All data used was abstracted from 3 routinely collected sources: the national surveillance system for RT-PCR test for Covid-19 (e-SUS Notifica); the information system for severe acute respiratory illness (SIVEP-Gripe) and the national immunisation system (SI-PNI).

e-SUS Notifica: This database contains information on suspected cases of Covid-19 recorded in the country. It includes all positive and negative RT-PCR test results, and information on residence, demographic and clinical data of individuals, such as presence of comorbidities and pregnancy status (so we can identify women registered during pregnancy) and presence of symptoms, with acute respiratory diseases defined as presence of at least two of the following signs and symptoms: fever (even if referred), chills, sore throat, headache, cough, runny nose, loss or change to a sense of smell or taste.[21] Asymptomatic individuals with a positive RT-PCR test confirming by Covid-19 infection are registered but were not included in this study.

SIVEP-Gripe is the national registration for severe acute respiratory syndrome (SARS) in Brazil, created after the Influenza pandemic of 2009. In 2020, it was expanded to include Covid-19. All Covid-19 hospitalisations and deaths are meant to be registered in this system.[22] In SIVEP-Gripe, severe acute respiratory illness is defined as an individual with acute respiratory disease who presents dyspnea/respiratory discomfort, persistent pressure or pain in the chest, oxygen saturation less than 95% without oxygen, or cyanosis of the lips or face.[22] Individuals who died with severe acute respiratory illness independent of hospitalisation are also registered. By linking these data with e-SUS Notifica, we identified which pregnant women in e-SUSNotify with a positive RT-PCR test progressed to severe disease.

SI-PNI contains data on all vaccines administered in Brazil. Covid-19 vaccines are administered by health services and recorded in point-of-care applications.[23] From SI-PNI, we extracted information on which Covid-19 vaccine was received with dates of first and second doses. By linking these data with the data on pregnant women in the other files, we were able to determine: (i) which pregnant women who tested negative for Covid-19 had been vaccinated (ii) which pregnant women with confirmed symptomatic Covid-19 infections had been vaccinated and (ii) which pregnant women with severe Covid-19 associated severe case had been vaccinated. We assumed that pregnant women whose record did not link to a SI-PNI vaccination record were not vaccinated.

All data were extracted on October 05, 2021 and made available by the Brazilian Ministry of Health. The information technology bureau of the Brazilian Ministry of Health provided pseudo-anonymised data with a common unique identifier that were used to link individual-level records from the three databases (more details about linkage procedures are available at https://vigivac.fiocruz.br/).

Study population

All pregnant women with symptoms suggesting Covid-19, aged between 18 and 49 years in Brazil with a record of a RT-PCR test between March 15, 2021, and October 03, 2021, registered in e-SUS Notifica. Testing for Covid-19 in Brazil is accessible to anyone through the universal public health system (SUS). Subjects who received any Covid-10 Vaccine were excluded: ChAdOx1 nCoV-19 or Ad26.COV2.S (Janssen/Johnson & Johnson) because these are not indicated for pregnant women in Brazil and BNT162b2 because numbers of women with complete regimen were too small to allow evaluation given they were included in the Brazilian program more recently and the long interval between doses. So, the study is restricted to evaluating CoronaVac vaccine effectiveness. The population consisted of symptomatic pregnant women who were tested with RT-PCR for Covid-19 classified into 3 groups: RT-PCR test negative, RT-PCR test positive with Covid-19 symptoms and RT-PCR test positive with severe Covid-19. The study population in the TND included all symptomatic women with a RT-PCR irrespective of test result. For the nested case control study only women in the first study who had a positive RT-PCR test for Covid-19.

Definition of outcome, cases, and controls

In the TND, the primary outcome was a positive RT-PCR test in a symptomatic subject. Cases were defined as all symptomatic women in the study population with a RT-PCR test result from a respiratory sample collected within 10 days after the onset of symptoms and who did not have a positive RT-PCR test result in the preceding 90 days. We also conducted an additional analysis for the subgroup of cases with severe Covid-19, identified through notification to SIVEP-Gripe or with a register of hospitalization or death in e-SUS record. Controls were defined as all women in the study population with a negative RT-PCR test result, and no positive RT-PCR test in the previous 90 days or in the subsequent 14 days. The test date was defined as either the date of collecting a respiratory specimen or the date of the case registration (when the test date was missing).

As a further consistency check, we estimated VE against progression from symptomatic Covid-19 disease to severe Covid-19 (severe, hospitalized or fatal) by comparing the vaccine status of those who developed severe disease with those who tested positive but did not develop

severe disease. Cases were defined as all women with severe Covid-19, identified through notification to SIVEP-Gripe or with a register of hospitalization or death in e-SUS record. Controls were defined as all confirmed cases of Covid-19 in e-SUS not notified to SIVEP-Gripe and with no registration of hospitalisation nor deaths in e-SUS.

Exposure definition

The exposure studied was vaccination with CoronaVac. This was classified into partially vaccinated (≥14 days after the first dose and before receipt of the second dose at time of RT-PCR testing) and fully vaccinated (≥14 days after the second dose at time of RT-PCR testing). We also calculated effectiveness in the period <14 days since vaccination as the vaccine is expected to have no or limited effectiveness in the first 13 days since vaccination. This was used as a test as high effectiveness or increased risk during this period might serve as an indicator of unmeasured bias or confounding. The reference group for vaccination status was the women who did not received a first vaccine dose before the date of sample collection.

Covariates

A number of risk factors may be associated with both the likelihood of the exposure (i.e., receiving a vaccine) and the likelihood of receiving an RT-PCR SARS-CoV-2 test. These include age, ethnicity, comorbidities status, geography location, index of deprivation,[24] and time (reflecting changes in vaccination policy and disease circulation) and presence of a previous Covid-19 positive RT-PCR as this may both related with vaccination and the risk of a second Covid-19 infection. We extracted information on these potential confounders from the e-SUS Notifica.

Statistical analyses

The test negative design is a type of case-control study, in which the study population consist of the population tested, and controls are selected from those who have a negative test. [25] Accordingly, both the test negative design and the additional comparison of severe cases with non-severe cases were analysed using the standard methods for case-control studies.[25,26] Logistic regression was used to estimate the odds of vaccination with CoronaVac in RT-PCR test confirmed cases compared with those who tested negative, and the odds of vaccination in the severe cases compared to those who tested negative; finally, we also estimated the odds of progression from symptomatic to severe Covid-19, by comparing the odds of vaccination in the severe cases to that in the non-severe cases. Individuals only contributed their first positive test result from March 15, 2021 (when the vaccination programme was recommended for pregnant women nationally). Week of RT-PCR test was included in the regression models because of the variations over time in both Covid-19 incidence and vaccine delivery in Brazil.

We also adjusted for age (<20, 20-34, ≥35), ethnicity (white, mixed brown, black and others), presence of registered comorbidities, geography (region), index of deprivation (quintile). We estimated the VE as one minus the corresponding odds ratio (OR), obtained from a model including the described covariates, expressed as a percentage.

Data analyses were performed in Stata version 17.0.

This study analysed de-identified data and was approved by the National Ethics committee (CONEP) (CAAE registration no. 50199321.9.0000.0040).

Results

During the study period, 95,738 symptomatic suspected cases of Covid-19 among pregnant women were registered in the Brazilian surveillance system e-SUS Notify. Of those, 50,819 (53.1%) had an RT-PCR SARS-CoV-2 test, and the results were available for 30,947 (60.9%) samples. After exclusions, 19838 subjects were included in the analysis; 7424 (37.4%) were test-positive, and 12414 (62.6%) test-negative. Of the 7424 with a positive test, 588 (7.9%) were severe and 84 (1.1%) died (Figure1). Table 1 shows the characteristics of cases and controls.

Figure 2 shows the number of cases and controls by time since the first and second vaccination doses among vaccinated pregnant women. After the first doses of CoronaVac, the proportion of positive tests does not seem to change. Notably, 165 (16.6%) out of all women with a single dose of CoronaVac had not received a second dose after the recommended interval between doses (4 weeks).

The odds of testing positive among vaccinated women during the 13 days after the first dose, was 1.35 (95% CI 1.09 to 1.68) compared with those unvaccinated, indicating an unexpected small increase in risk of Covid-19 among the vaccinated during this initial period. VE among those receiving only the first dose with at least 14 days between the first dose and the date of RT-PCR) was low and not statistically significant 5.02 (95% CI -18.22- 23.69). The estimated adjusted VE in the fully vaccinated group against symptomatic Covid-19 was 41.0% (95% CI 27.1 to 52.2) (Table 2). The corresponding estimate for severe Covid-19 was 67.7 (95% CI 20.0-87.0) for those partially vaccinated and 85.4 (95% CI 59.4- 94.8) for fully vaccinated women (Table 3).

The estimated adjusted VE of CoronaVac against progression from symptomatic to severe Covid-19 was 67.4% (95% CI 17.7 to 87.1) among partially vaccinated pregnant women and 74.7% (95% CI 28.0 to 91.2) among fully vaccinated women (Table 3). No deaths occurred

among partially or fully vaccinated pregnant women when four would have been expected if mortality was the same as in unvaccinated.

Discussion

In this investigation of CoronaVac VE in pregnant women, we found that a single dose of the CoronaVac vaccine offered no protection against symptomatic Covid-19; two doses were 41% effective against symptomatic Covid-19 and 85% effective against severe Covid-19. Those who were fully vaccinated and went on to have symptoms had a 75% lower risk of progressing to severe Covid-19 than those unvaccinated. No deaths occurred among partially or fully vaccinated women, when 4 were expected. About 17% of vaccinated women did not get a second dose as prescribed by the time they were tested.

Although the findings from this study suggest that the complete CoronaVac vaccine regimen was effective against symptomatic Covid-19 among pregnant women, the magnitude of estimated effectiveness was lower than reported previously in studies in the general population conducted in Brazil,[8] Chile,[5] and Turkey.[27] Pregnancy promotes resistance to generating proinflammatory antibodies compared to non-pregnant women, suggesting that pregnant women may not respond to some vaccines as effectively.[28,29] We did not investigate biological mechanisms; further investigation is required to establish whether the lower effectiveness found is due to immunological changes during pregnancy. In contrast with other Covid-19 vaccines such as the BNT162b2 which confers protection after the first dose,[30] CoronaVac was effective against symptomatic Covid -19 only after a complete regimen. This was also found is in older people in Brazil.[31]

This study has strengths and limitations. As a strength, it used rich, routinely collected data from Brazil, recognised to be of high-quality.[32] By using the TND, we have minimised bias related to access to health care, the occurrence of symptoms and health-seeking behaviour. In most populations strong pressures have influenced who got tested for Covid-19. These biases can mean that those who get tested, and test positive for SARS-CoV-2 may not be a random sample of all cases in the population. The assumption that underlies the TND is that people who seek testing and manage to get tested would be influenced by similar pressures regardless of vaccine status and the test outcome,[26] thus biases will 'cancel out' and relatively unbiased estimates of effect can be obtained.[25,26]

However, as observational designs are vulnerable to confounding and bias. The fact that the risk of Covid-19 increased in vaccinated women in the 2 weeks after the first dose is not biological plausible and may be an indication of residual bias/confounding, which in this

case could lead to an underestimation of VE. A potential explanation for this would be if vaccinated subjects feel safer than unvaccinated subjects, such that unvaccinated subjects are more likely to seek testing for a symptom (not caused by Covid-19) that would not lead a vaccinated subject to test. This would result in a higher proportion of negative tests among the unvaccinated, leading to an apparent estimated increase in risk in the vaccinated, underestimating VE. Other potential explanations are that the process of vaccination itself increases the risk of infection, such travelling to or from a vaccination site, and finally, that after being vaccinated, believing themselves to be protected, women undergo a period of 2 weeks of contacts and reduced protective measures, leading to a peak of infection shortly after vaccination.

A limitation intrinsic to the use and availability of secondary data is the limited choice of covariates and the potential for misclassifying vaccine status due to linkage failure. Finally, we did not assess vaccination safety as data necessary for this assessment was not available. However, it is reassuring that CoronaVac contains an adjuvant that is commonly used in many other vaccines, such as against Hepatitis B and Tetanus, with a well-documented safety profile among pregnant women.[34] Previous evidence of safety of inactivated vaccines for other pathogens and using this adjuvant is reassuring.[34]

We note that an alarming 17% of the study sample with a single dose of CoronaVac did not take the second dose after the recommended maximum interval (4 weeks). This has important repercussions for public health authorities, highlighting the importance of actively searching those delaying the second doses and promoting opportunities to vaccinate these women during regular prenatal care appointments.

In conclusion, this study involved pregnant women in a setting that combines high disease burden and elevated Covid-19 related maternal related deaths. In this setting, we found that a complete regimen of CoronaVac was 41% effective in preventing symptomatic Covid-19, and 85% effective in preventing severe Covid-19 disease; it was 75% effective in preventing severe outcomes in those who had been infected.

Contributors
ESP, NP, MLB, MBN developed the study concept. VAO, TCS, JBJ, TMM, GP, MBN acquired, treated and linked the data. KLMW, FJOA, EPPJ, VO, GLW, LCR contributed to the data analyses and interpretation of results. VAO, KLMW vouched for the data analyses. ESP wrote the first draft. All authors decided to publish and revised the manuscript and approved the final version.

Declarations
We declare no competing interests. VO, VB, MB, and MB-N are employees from Fiocruz, a federal public institution, which manufactures Vaxzevria in Brazil, through a full technology transfer agreement with AstraZeneca. Fiocruz allocates all its manufactured products to the Ministry of Health for the public health service (SUS) use.

Funding
This study is part of the VigiVac Fiocruz program, partially supported by a donation from the "Fazer o bem faz bem" program. EPS is funded by the Wellcome Trust [Grant number 213589/Z/18/Z]. The funders had no role in study design, analysis, decision to publish or preparation of the manuscript.

This research was funded in part by the Wellcome Trust. For the purpose of open access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

Acknowledgments
GLW, MLB, and MB-N are research fellows from CNPq, the Brazilian National Research Council.

References

1. Abu-Raya B, Michalski C, Sadarangani M, Lavoie PM. Maternal immunological adaptation during normal pregnancy. Frontiers in Immunology 2020,11, 2627.
2. Allotey J, Stallings E, Bonet M, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis.Bmj 2020, 370.
3. BioNTech, S. E. Study to Evaluate the Safety, Tolerability, and Immunogenicity of SARS CoV-2 RNA Vaccine Candidate (BNT162b2) Against COVID-19 in Healthy Pregnant Women 18 Years of Age and Older.2021
4. Duarte G, Coutinho CM, Rolnik DL, Quintana SM, Rabelo e Silva AC,Poon LC et al..Perspectives on administration of COVID-19 vaccine to pregnant and lactating women: a challenge for low-and middle-income countries. AJOG Global Reports 2021: 100020.
5. Jara A, Undurraga EA, González C J, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. N Engl J Med 2021;385:875-884
6. Thompson MG, Stenehjem E, Grannis S, et al. Effectiveness of Covid-19 vaccines in ambulatory and inpatient care settings. N Engl J Med 2021;385:1355-1271.
7. Lopez Bernal J, Andrews N, Gower C, Robertson C, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. BMJ. 2021 May 13;373:n1088. doi: 10.1136/bmj.n1088. PMID: 33985964; PMCID: PMC8116636.
8. Ranzani O T, Hitchings M D T, Dorion M, et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study. BMJ 2021; 374 :n2015 doi:10.1136/bmj.n2015
9. Dagan N, Barda N, Biron-Shental T, et al. Effectiveness of the BNT162b2 mRNA COVID-19 vaccine in pregnancy. Nat Med. 2021 Oct;27(10):1693-1695. doi: 10.1038/s41591-021-01490-8. Epub 2021 September 07. PMID: 34493859.
10. Goldshtein I, Nevo D, Steinberg DM, et al. Association Between BNT162b2 Vaccination and Incidence of SARS-CoV-2 Infection in Pregnant Women. JAMA. 2021 August 24;326(8):728-735. doi: 10.1001/jama.2021.11035. PMID: 34251417; PMCID: PMC8276131.
11. Bleicher I, Kadour-Peero E, Sagi-Dain L, Sagi S. Early exploration of COVID-19 vaccination safety and effectiveness during pregnancy: interim descriptive data from a prospective observational study. Vaccine. 2021 Oct 22;39(44):6535-6538. doi: 10.1016/j.vaccine.2021.09.043. Epub 2021 September 25. PMID: 34600749; PMCID: PMC8463327.
12. Butt AA, Chemaitelly H, Al Khal A, et al. SARS-CoV-2 vaccine effectiveness in preventing confirmed infection in pregnant women. J Clin Invest. 2021 Oct 6:e153662. doi: 10.1172/JCI153662. Epub ahead of print. PMID: 34618693.
13. Dagan N, Barda, Biron-Shental N et al. Effectiveness of the BNT162b2 mRNA COVID-19 vaccine in pregnancy. Nature Medicine 27.10 (2021): 1693-1695.
14. Brazilian Ministry of Health. Campanha Nacional de Vacinação contra a Covid-1. Décimo oitavo informe técnico.(https://www.conasems.org.br/wp-content/uploads/2021/04/20adistribuic%CC%A7a%CC%83o_Campanha_Nacional_de_Vacinacao_contra_a_Covid_19.pdf)
15. Brazilian Ministry of Health. Nota Técnica Nº 1/2021-DAPES/SAPS/MS - Vaccination for pregnant and postpartum women with comorbities.2021(https://portaldeboaspraticas.iff.fiocruz.br/wpcontent/uploads/2021/03/NT-vacinacao-gestantes-peurperas-e-lactantes.pdf)

16. Brazilian Ministry of Health. Nota Técnica Nº 467/2021-CGPNI/DEIDT/SVS/MS - Vaccination for pregnant and postpartum women without comorbidities.2021(https://portaldeboaspraticas.iff.fiocruz.br/wpcontent/uploads/2021/04/nota-tecnica-467-2021-cgpni-deidt-svs-ms.pdf)
17. Brazilian Ministry of Health. COVID-19 Vacinação Doses Aplicadas. (https://qsprod.saude.gov.br/extensions/demas_c19vacina/demas_c19vacina.html)
18. Worldmeters. Coronavirus update (Live) COVID-19 virus pandemic. 2021. (https://www.worldometers.info/coronavirus/)
19. de Carvalho, RC, Costa MCN, Teixeira MG, et al. "Impact of COVID-19 pandemic on time series of maternal mortality ratio in Bahia, Brazil: analysis of period 2011–2020." BMC Pregnancy and Childbirth 2021, 21.1: 1-7.
20. Takemoto MLS, Menezes MO, Andreucci CB, Nakamura-Pereira M, Amorim MMR, Katz L, Knobel R. The tragedy of COVID-19 in Brazil: 124 maternal deaths and counting. Int J Gynaecol Obstet. 2020 Oct;151(1):154-156. doi: 10.1002/ijgo.13300. Epub 2020 July 29. PMID: 32644220.
21. Brazilian Ministry of Health. e-SUS Notifica. https://datasus.saude.gov.br/notifica/(accessed october, 2021)
22. Brazilian Ministry of Health. Opendatasus. https://opendatasus.saude.gov.br/dataset/bd-srag-2021. (accessed october, 2021)
23. Brazilian Ministry of Health. SI-PNI - Sistema de Informação do Programa Nacional de Imunizações. http://pni.datasus.gov.br/. (accessed october, 2021)
24. Allik M, Ramos D, Agranonik M et al. Developing a Small-Area Deprivation Measure for Brazil. Technical Report. Cidacs/University of Glasgow. 2020. https://cidacs.bahia.fiocruz.br/ibp/wp-content/uploads/2020/12/technical-report_compressed.pdf
25. Vandenbroucke JP, Pearce N. Test-Negative Designs: Differences and Commonalities with Other Case-Control Studies with "Other Patient" Controls. Epidemiology. 2019 Nov;30(6):838-844. doi: 10.1097/EDE.0000000000001088. PMID: 31430265.
26. Vandenbroucke JP, Brickley EB, Vandenbroucke-Grauls CMJE, Pearce N. A Test-Negative Design with Additional Population Controls Can Be Used to Rapidly Study Causes of the SARS-CoV-2 Epidemic. Epidemiology. 2020 Nov;31(6):836-843. doi: 10.1097/EDE.0000000000001251. PMID: 32841988; PMCID: PMC7523580.
27. Tanriover, M D, Doğanay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey.The Lancet.2021: 398.10296: 213-
28. Atyeo, C, DeRiso EA, Davis C et al. COVID-19 mRNA vaccines drive differential Fc-functional profiles in pregnant, lactating, and non-pregnant women. *bioRxiv* 2021.
29. Saeed Z, Greer O, Shah NM. Is the Host Viral Response and the Immunogenicity of Vaccines Altered in Pregnancy? Antibodies (Basel). 2020 Aug 4;9(3):38. doi: 10.3390/antib9030038. PMID: 32759839; PMCID: PMC7551810.
30. Roest S, Hoek RAS, Manintveld OC. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. N Engl J Med. 2021 May 20;384(20):1968-1970. doi: 10.1056/NEJMc2104281. Epub 2021 April 21. PMID: 33882226.
31. Ranzani OT, Hitchings MDT, Dorion M, D'Agostini TL, de Paula RC, de Paula OFP, Villela EFM, Torres MSS, de Oliveira SB, Schulz W, Almiron M, Said R, de Oliveira RD, Vieira da Silva P, de Araújo WN et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study. BMJ. 2021 Aug 20;374:n2015. doi: 10.1136/bmj.n2015.

32. Jorge MH P M, Laurenti R, Gotlieb SLD. Análise da qualidade das estatísticas vitais brasileiras: a experiência de implantação do SIM e do SINASC. *Ciência & Saúde Coletiva* 12. 2007
33. MHPM Jorge, R Laurenti, SLD Gotlieb. Análise da qualidade das estatísticas vitais brasileiras: a experiência de implantação do SIM e do SINASC. Ciência & Saúde Coletiva, v. 12, p. 643-654, 2007.
34. WHO. The Sinovac-CoronaVac COVID-19 vaccine: What you need to know. September 2021 (https://www.who.int/news-room/feature-stories/detail/the-sinovac-covid-19-vaccine-what-you-need-to-know)

Figure 1: Flowchart of the study population from surveillance system and final sample of cases and controls

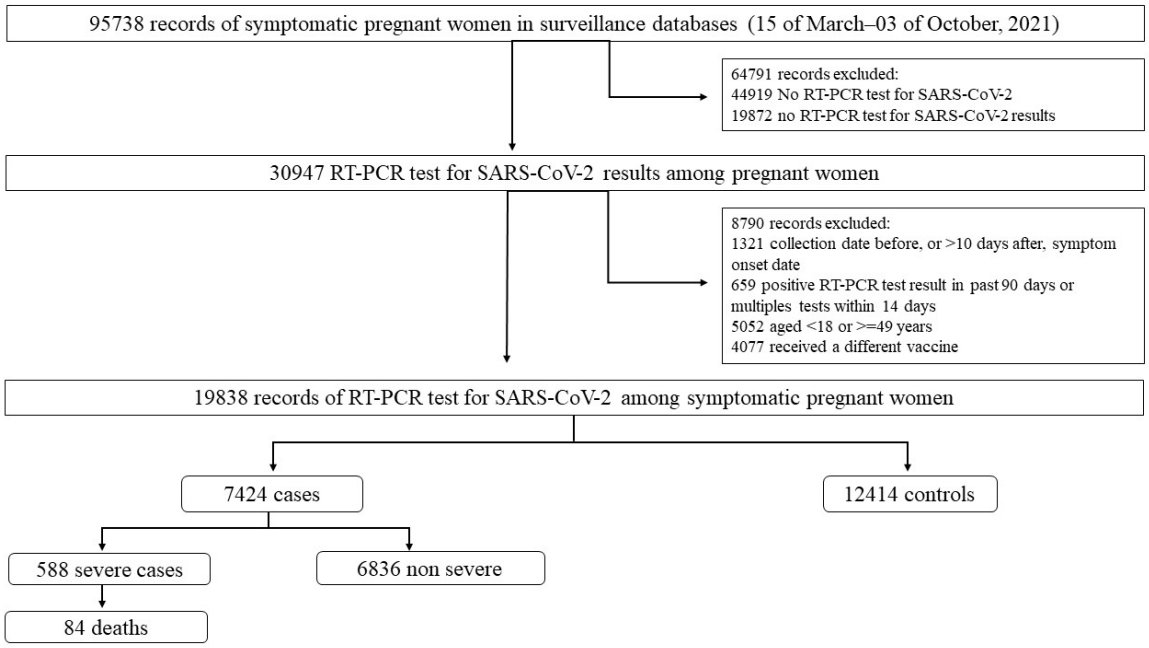


Figure 2: Number of cases and controls by interval since first and second vaccination

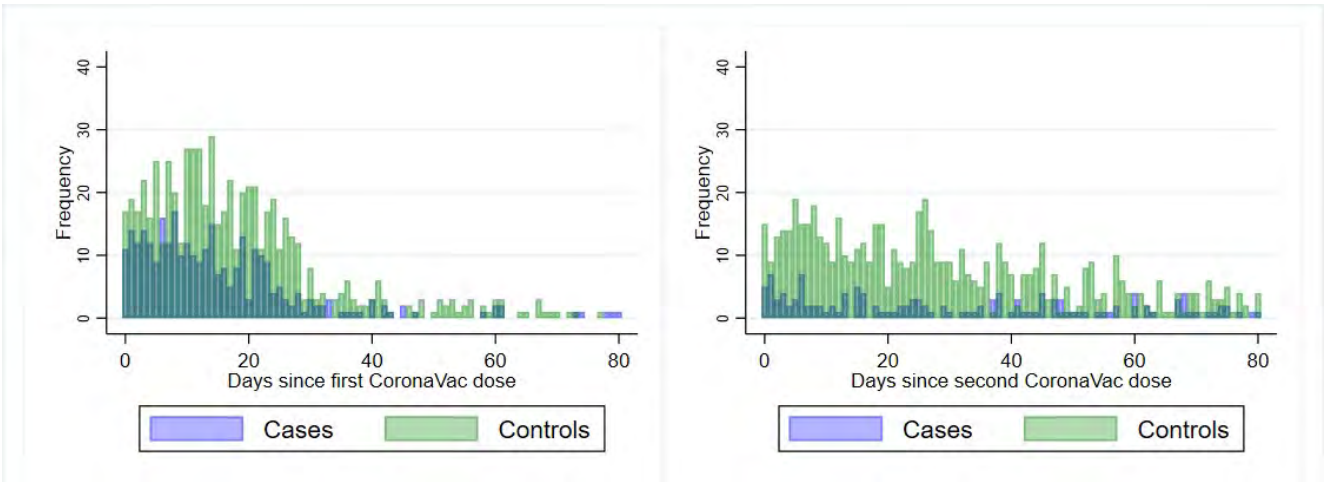


Table 1: Characteristics of cases and controls in pregnant women aged 18-49 years in Brazil.

Characteristics	Test positive	Test negative
Vaccination status		
Not vaccinated	6886 (92.75)	10919 (87.96)
Single dose, within 0-13 days	169 (2.28)	284 (2.29)
Single dose, ≥14 days	156 (2.10)	386 (3.11)
Two doses, within 0-13 days	45 (0.61)	192 (1.55)
Two doses, ≥14 days	168 (2.26)	633 (5.10)
Age group		
< 20	406 (5.47)	940 (7.57)
20-34	5606 (75.51)	9629 (77.57)
35+	1412 (19.02)	1845 (14.86)
Missing	-	-
Self-reported race		
White	2787 (43.75)	5226 (47.93)
Mixed Brown	3085 (48.43)	4830 (44.30)
Black	390 (6.12)	689 (6.32)
Others	108 (1.70)	158 (1.45)
Missing	1054	1511
Reported co-morbidities		
Yes	554 (7.46)	767 (6.18)
No	6870 (92.54)	11647 (93.82)
Missing*	-	-
Previous events notified to surveillance		
Yes	2447 (32.96)	5145 (41.45)
No	4977 (67.04)	7269 (58.55)
Missing	-	-
Brazilian Deprivation Index		
1	1940 (26.13)	3634 (29.29)
2	1638 (22.07)	2949 (23.77)
3	1502 (20.23)	2269 (18.29)
4	1293 (17.42)	2039 (16.43)
5	1050 (14.15)	1518 (12.23)
Missing	1	5
Region of residence		
North	349 (4.70)	623 (5.02)
Northeast	1663 (22.40)	2244 (18.08)
South	734 (9.89)	2136 (17.21)
Southeast	3981 (53.62)	6444 (51.92)
Midwest	697 (9.39)	965 (7.77)
Missing	-	2

* those who reported only pregnancy as condition were considered without co-morbidities

Table 2: Effectiveness of -CoronaVac against symptomatic and severe Covid-19, among pregnant women aged 18-49 years in Brazil (comparison of symptomatic and severe cases with test-negative controls)

	Unadjusted Odds Ratio (95% CI)	Unadjusted# Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	Adjusted* VE% (95% CI)	p-value
Vaccination status					
Symptomatic Covid-19	**Sinovac-CoronaVac**				
Unvaccinated	Ref	Ref	Ref	Ref	
One dose <13 days	0.94 (0.77-1.14)	1.35 (1.10-1.66)	1.35 (1.09-1.68)	-	0.006
Partially vaccinated (One dose ≥14 days)	0.64 (0.53-0.77)	1.00 (0.82-1.22)	0.94 (0.76-1.18)	5.02 (-18.22- 23.69)	0.645
Two doses ≥14 days	0.42 (0.35-0.50)	0.69 (0.57-0.83)	0.59 (0.47-0.72)	40.97 (27.07- 52.22)	<0.001
Severe Covid-19					
Unvaccinated	Ref	Ref	Ref	Ref	
One dose <13 days	1.38 (0.87-2.19)	1.64 (1.01-2.65)	1.42 (0.83-2.43)	-	0.192
Partially vaccinated (One dose ≥14 days)	0.30 (0.13-0.69)	0.38 (0.16-0.87)	0.32 (0.13-0.80)	67.74 (20.00-87.00)	0.015
Two doses ≥14 days	0.15 (0.06-0.37)	0.20 (0.08-0.50)	0.14 (0.05-0.40)	85.39 (59.44- 94.80)	<0.001

Table 3: Effectiveness of Sinovac-CoronaVac against symptomatic Covid-19 and progressing to severe forms (comparing severe, hospitalized or fatal Covid-19 with test negative), among pregnant women aged 18-49 years in Brazil (comparison of severe cases with non-severe cases)

	Unadjusted Odds Ratio (95% CI)	Unadjusted# Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	Adjusted* VE% (95% CI)	p-value
Vaccination status					
Symptomatic Covid-19	**Sinovac-CoronaVac**				
Severe Covid-19					
Unvaccinated	Ref	Ref	Ref	Ref	
One dose <13 days	1.52 (0.95-2.45)	1.16 (0.70-1.93)	1.02 (0.58-1.78)	-	0.932
Partially vaccinated (One dose ≥14 days)	0.45 (0.20-1.04)	0.34 (0.15-0.80)	0.32 (0.12-0.82)	67.46 (17.66- 87.14)	0.018
Two doses ≥14 days	0.35 (0.14-0.86)	0.27 (0.10-0.69)	0.25 (0.08-0.72)	74.69 (27.95-91.20)	0.001

Supplementary material

Table S1: Vaccination plan for pregnant and postpartum women in Brazil

Date	Technical notes issued by the Ministry of Health	Recommendations
15/03/2021	**NOTA TÉCNICA Nº 1/2021-DAPES/SAPS/MS -** Vaccination for pregnant and postpartum women with comorbities	- Vaccination for pregnant and lactating women with comorbidities - Vaccine can be offered to pregnant and postpartum women without comorbidities after evaluating the risks and benefits, especially considering the professional activity performed by the woman.
26/04/2021	**NOTA TÉCNICA Nº 467/2021-CGPNI/DEIDT/SVS/M**S - Vaccination for pregnant and postpartum women without comorbidities	Phase I- Pregnant and postpartum women with comorbidities, regardless of age Phase II- Pregnant and postpartum women, regardless of comorbidities
14/05/2021	**NOTA TÉCNICA nº 627/2021-CGPNI/DEIDT/SVS/MS -** Temporary suspension of vaccination	- Temporary suspension of vaccination with the vaccine AstraZeneca/Oxford/Fiocruz in pregnant and postpartum women
19/05/2021	**NOTA TÉCNICA Nº 651/2021 - CGPNI/DEIDT/SVS/MS - C**ontinued vaccination in pregnant and postpartum women with comorbidities	- Vaccination of pregnant and postpartum women with comorbidities after benefit risk evaluation and medical prescription (Vaccines without viral vector -SINOVAC/Butantan or Pfizer-BioNTech BNT162b2) - Pregnant and postpartum women (including those without additional risk factors) who have already received the first dose of the AstraZeneca/Oxford/Fiocruz vaccine must wait for the end of the gestation and postpartum period (up to 45 days after delivery) for the administration of the second dose of the vaccine - Pregnant and postpartum women (including those without additional risk factors) who have already received the first dose of another COVID-19 vaccine that does not contain a viral vector (Sinovac/Butantan or Pfizer-BioNTech BNT162b2) should complete the regimen with the same vaccine at the usual intervals - Pregnant and postpartum women of other priority groups (health workers or other essential services workers, for example) may be vaccinated after an individual risk and benefit evaluation
06/07/2021	**NOTA TÉCNICA Nº 2/2021 - SECOVID/GAB/SECOVID/MS -** Continued vaccination in pregnant and postpartum women without comorbidities	- Vaccination of pregnant and postpartum women aged 18 years and over, regardless of risk factors - Pregnant of any gestational age - Needs for Medical evaluation and Prescription
23/07/2021	**NOTA TÉCNICA Nº 6/2021-SECOVID/GAB/SECOVID/MS -** Interchangeability between vaccines for pregnant and postpartum women who	- Vaccination of pregnant and postpartum women aged 18 years and over, regardless of risk factors - Pregnant of any gestational age

	took the oxford astrazeneca vaccine in the first dose	- Need for Medical evaluation and Prescription - To pregnant and postpartum women who received the first dose of the AstraZeneca/Fiocruz vaccine, at time of the second dose, preferably, the Pfizer-BioNTech BNT162b2 /Wyeth vaccine should be offered. If this immunising agent is not available locally, Sinovac/Butantan vaccine may be used

3.2. Proteção contra a Covid-19 gerada pela CoronaVac é passada aos bebês pelo leite materno das mães, aponta pesquisa

Um estudo feito pelo Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCF-MUSP) aponta que lactantes que receberam a CoronaVac, vacina produzida pelo Instituto Butantan em parceria com a farmacêutica chinesa Sinovac, apresentam anticorpos contra Covid-19 no leite materno, capazes de proteger também os bebês, até quatro meses após a vacinação.

A pesquisa foi realizada com 20 funcionárias que foram imunizadas entre janeiro e fevereiro de 2021. Foram recolhidas nove amostras de leite no total: antes da imunização, quatro vezes depois da primeira dose e três vezes após a segunda dose, com intervalos de sete dias e quatro meses após a vacinação.
A pesquisa mostrou que os níveis de anticorpos do leite materno ainda estavam altos quatro meses após a vacinação. Os auges da produção de anticorpos se deram na segunda semana após a primeira dose e na quinta e na sexta semana após a segunda dose.

A imunização das lactantes e gestantes oferece proteção de duas formas: aos bebês ainda não nascidos, por meio da placenta, com anticorpos IgG, e por meio do leite materno, aos recém-nascidos, com anticorpos IgA.

De acordo com o Ministério da Saúde, cerca de 500 mil grávidas e puérperas com comorbidades já foram vacinadas contra a Covid-19 no Brasil. As gestantes se tornaram público prioritário da campanha de vacinação porque a taxa de letalidade da Covid-19 entre elas é muito maior que a média (10% para grávidas contra 2% da população em geral). Apenas duas vacinas são recomendadas para as gestantes, sendo uma delas a CoronaVac, por ter grande eficácia e um alto perfil de segurança.

Publicado em: 23/06/2021

CLINICS

EDITORIAL

CoronaVac can induce the production of anti-SARS-CoV-2 IgA antibodies in human milk

Valdenise Martins Laurindo Tuma Calil,[I,#] **Patricia Palmeira**,[II,*,#] **Yingying Zheng**,[III] **Vera Lúcia Jornada Krebs**,[I] **Werther Brunow de Carvalho**,[III] **Magda Carneiro-Sampaio**[II,III]

[I] Instituto da Crianca e do Adolescente, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR. [II] Laboratorio de Pediatria Clinica (LIM36), Departamento de Pediatria, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR. [III] Departamento de Pediatria, Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, SP, BR.

Calil VMLT, Palmeira P, Zheng Y, Krebs VLJ, Carvalho WB, Carneiro-Sampaio M. CoronaVac can induce the production of anti-SARS-CoV-2 IgA antibodies in human milk. Clinics (Sao Paulo). 2021;76:e3185

*Corresponding author. E-mail: patricia.palmeira@hc.fm.usp.br
[#]Contributed equally as co-first authors.

To the Editor,

Human milk is the external secretion with the highest immunoglobulin A (IgA) concentrations, mostly produced in the lamina propria of mammary glands by plasma cells (1). The milk antibody repertoire is quite similar to the one observed in the blood; however, the levels of antibodies against enteric and respiratory pathogens are usually higher in the colostrum and mature milk than in the serum. Maternal immunization can elicit systemic immunoglobulin G (IgG) and mucosal IgA, IgM, and IgG responses that confer protection to the newborn infants (2,3,4).

During the current pandemic, milk anti-SARS-CoV-2-specific IgA antibodies have been found in 23.1% of 2,312 previously infected lactating women (5,6). In an Israeli prospective cohort, milk samples of 84 breastfeeding women were analyzed before immunization and then weekly for six weeks after immunization. All the mothers received two doses of the Pfizer-BioNTech vaccine 21 days apart (7). The levels of IgA antibodies were significantly elevated two weeks after the first dose, with 61.8% of the samples testing positive (86.1% at week 4—one week after the second dose, and 65.7% at week 6).

Here, we present data from an initial study on the presence of anti-SARS-CoV-2 IgA antibodies in human milk samples obtained from volunteers during the immunization process promoted by HC-FMUSP in January (17^{th}-21^{st}) and February (15^{th}-18^{th}), 2021. The preparation "CoronaVac" (an inactivated vaccine), produced by Sinovac Biotech Ltd. (China) and Instituto Butantan (Brazil), was administered to all healthy employees in two doses, four weeks apart. A total of 170 samples were collected. All the 20 milk donors were HC-FMUSP employees and were breastfeeding at the time of the first immunization phase and voluntarily donated 5-10 mL milk samples before the first dose and seven more samples weekly for three weeks after the second dose. Milk samples were collected four months after the first dose from 10 mothers to evaluate the persistence of SARS-CoV-2-specific IgA antibodies. Milk was collected by the donors themselves into sterile containers after careful local antisepsis with sterile water. Manual expression or milk pump were used for sample collection after rigorous handwashing. The milk was stored at home by the donor at -20ºC until delivery to the laboratory (LIM-36-ICr).

The study was approved by the Institutional Ethics Board (CAAE: 45565121.2.0000.0068), and written informed consent was obtained from all the participants. The levels of IgA antibodies that specifically bind the S1 domain of the spike protein (including RBD-Receptor Binding Domain) were semiquantitatively analyzed using the Euroimmun anti-SARS-CoV-2 S1 ELISA kit. The results were presented as the ratio of the optical density of the samples and the optical density of the calibrator (both read at 450 nm, using a reference wavelength of 620 nm), and ratios above 0.8 were considered positive. One-way ANOVA followed by Tukey's multiple comparison tests were used in the statistical analysis (GraphPad v.7.0 Software Inc., San Diego, CA, USA), and statistical significance was set at $p<0.05$.

No significant adverse reactions were reported in either the mothers or their babies. The mean maternal age was 35.6 (±3.2) years at the time of the first dose, with a mean nursing period of 11.2 (±8.7) months, quite similar to the Israeli study, which was 10.3 months (7).

Of the 20 mothers, 16 were COVID-negative at week 0 (Figure 1). Despite an increase in the mean levels of anti-SARS-CoV-2-specific IgA in the first two weeks after the first dose, significantly higher mean values were obtained only at weeks 5 and 6. Ten mothers presented specific IgA antibody levels above the seroconversion value at week 7 (21 days after the second dose). Among the ten mothers who donated a sample four months after the first dose, five still had specific IgA levels above the seroconversion value at that time. In our series, four mothers had COVID-19, of whom three presented high levels of anti-SARS-CoV-2 IgA antibodies in W0 (data not shown). One of them donated her milk four months after the first vaccine dose and still had high specific IgA levels (anti-SARS-CoV-2-specific IgA ratio=4.0).

No potential conflict of interest was reported.

Received for publication on May 26, 2021. **Accepted for publication on** June 10, 2021

DOI: 10.6061/clinics/2021/e3185

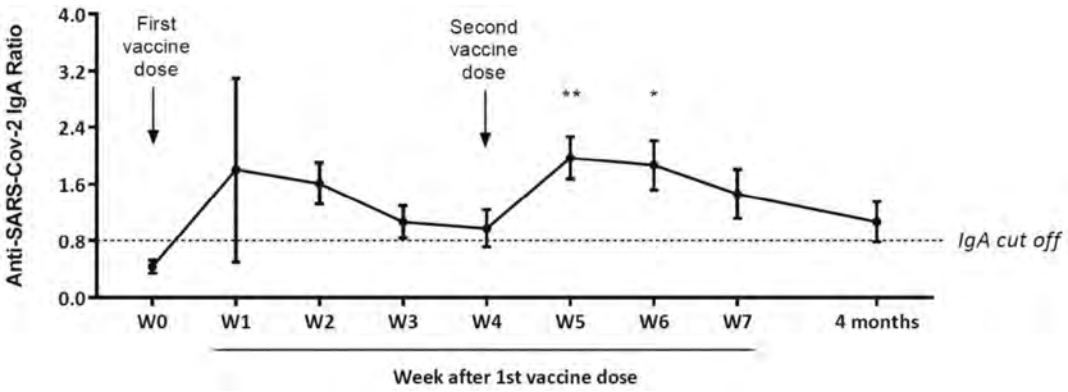


Figure 1 - Anti-SARS-CoV-2-specific IgA ratios (mean ± standard error) in milk samples collected over time (Weekly–W) from 16 healthy mothers previously COVID-negative after a 2-dose schedule of the CoronaVac vaccine (Sinovac Biotech Ltd., China). The last withdrawal was performed four months after the first dose in ten mothers. $^{**}p < 0.01$; $^{*}p < 0.05$.

This study strongly reinforces that mothers should continue breastfeeding their children after vaccination against SARS-CoV-2 and even after infection (5-7). As for other respiratory infections, maternal anti-SARS-CoV-2 immunization should protect infants with systemic IgG and milk IgA providing local mucosal defense, as demonstrated by Gray et al. (8) in a large group of pregnant and lactating women who received Pfizer-BioNTech vaccine where all cord blood and breastmilk samples presented specific IgG and IgA antibodies, respectively. Therefore, to analyze both the placental transfer of anti-SARS-CoV-2 IgG and production of IgA in early milk, we are planning an equivalent protocol with "CoronaVac" immunization during pregnancy involving the collection of maternal and cord blood, colostrum, and milk during the first two post-delivery months (3,4).

ACKNOWLEDGMENTS

We are grateful to all the participating mothers, to the press officer Markione de Santana Assis for logistical assistance during the research, nutritionists Analisa Gabriela, Zucchi Leite, and Renata Hyppolito Barnabe for assistance in the collection and transport of milk samples, and to FAPESP (grant 2014/50489-9) for Euroimmun anti-SARS-CoV-2 S1 ELISA kit acquisition.

AUTHOR CONTRIBUTIONS

Calil VMLT, Palmeira P, and Carneiro-Sampaio M contributed substantially to the study conception and design, data analysis and interpretation, manuscript writing and editing. Zheng Y was responsible for sample collection, laboratory, and statistical analyses. Krebs VLJ and Carvalho WB were responsible for revising the manuscript. All of the authors critically revised the manuscript and approved its final version.

REFERENCES

1. Palmeira P, Carneiro-Sampaio M. Immunology of breast milk. Rev Assoc Med Bras (1992). 2016;62(6):584-93. https://doi.org//10.1590/1806-9282.62.06.584
2. Maertens K, De Schutter S, Braeckman T, Baerts L, Van Damme P, De Meester I, et al. Breastfeeding after maternal immunisation during pregnancy: providing immunological protection to the newborn: a review. Vaccine. 2014;32(16):1786-92. https://doi.org/10.1016/j.vaccine.2014.01.083
3. Faucette AN, Unger BL, Gonik B, Chen K. Maternal vaccination: moving the science forward. Hum Reprod Update. 2015;21(1):119-35. https://doi.org/10.1093/humupd/dmu041
4. Lima L, Molina MDGF, Pereira BS, Nadaf MLA, Nadaf MIV, Takano OA, et al. Acquisition of specific antibodies and their influence on cell-mediated immune response in neonatal cord blood after maternal pertussis vaccination during pregnancy. Vaccine. 2019;37(19):2569-79. https://doi.org/10.1016/j.vaccine.2019.03.070
5. Calil VMLT, Krebs VLJ, Carvalho WB. Guidance on breastfeeding during the Covid-19 pandemic. Rev Assoc Med Bras (1992). 2020;66(4):541-6.
6. Juncker HG, Romijn M, Loth VN, Ruhé EJM, Bakker S, Kleinendorst S, et al. Antibodies Against SARS-CoV-2 in Human Milk: Milk Conversion Rates in the Netherlands. J Hum Lact. 2021;8903344211018185. https://doi.org/10.1177/08903344211018185
7. Perl SH, Uzan-Yulzari A, Klainer H, Asiskovich L, Youngster M, Rinott E, et al. SARS-CoV-2–Specific Antibodies in Breast Milk After COVID-19 Vaccination of Breastfeeding Women. JAMA. 2021;325(19):2013-4. https://doi.org/10.1001/jama.2021.5782
8. Gray KJ, Bordt EA, Atyeo C, Deriso E, Akinwunmi B, Young N, et al. Coronavirus disease 2019 vaccine response in pregnant and lactating women: a cohort study. Am J Obstet Gynecol. 2021;S0002-9378(21)00187-3. https://doi.org/10.1016/j.ajog.2021.03.023

CoronaVac

O que a ciência comprova

4. Protege indivíduos com comorbidades

4.1. CoronaVac é segura e eficaz em pacientes com risco aumentado para trombose, diz estudo

Uma pesquisa conduzida em São Paulo com indivíduos com alto risco de trombose, portadores de uma doença autoimune chamada síndrome antifosfolípide, mostrou que a CoronaVac é uma vacina segura e eficaz para esse público. O trabalho foi publicado na revista Lupus por cientistas da Faculdade de Medicina da Universidade de São Paulo (FMUSP) e coordenado pela pesquisadora e médica reumatologista Eloisa Bonfá, diretora clínica do Hospital das Clínicas da FMUSP.

Relatos recentes no exterior de casos de trombose em pessoas que haviam tomado vacinas de adenovírus chamaram a atenção da comunidade científica. Para avaliar esse risco no caso da CoronaVac, vacina de vírus inativado, os pesquisadores selecionaram 44 pacientes com síndrome antifosfolípide e 132 controles, com idade média de 46 anos. Todos os participantes receberam duas doses da CoronaVac.

Em relação à quantidade de anticorpos antifosfolípides em pacientes com a síndrome, não foi encontrada diferença significativa entre as amostras coletadas antes e depois da vacinação, mostrando que a CoronaVac é segura e não influencia na doença. Os voluntários foram acompanhados durante seis meses e nenhum evento trombótico foi observado nesse período.

"Indivíduos com a síndrome frequentemente apresentam comorbidades como hipertensão, obesidade e dislipidemia, que também podem favorecer eventos coagulantes, mas isso não aconteceu em nenhum caso", reforçam os pesquisadores no artigo.

A vacina também se mostrou altamente imunogênica. Seis semanas após a segunda dose, ambos os grupos apresentaram soroconversão elevada e comparável, com produção de anticorpos IgG em 83,9% dos pacientes e 93,5% dos controles. Os pacientes também apresentaram alta positividade para anticorpos neutralizantes (77,4%), com atividade de 64,3%. Não foram observados efeitos adversos graves nem moderados.

Segundo os cientistas, os achados de segurança e imunogenicidade apoiam a recomendação da CoronaVac para os pacientes com síndrome antifosfolípide.

Publicado em: 22/5/2022

Paper

Lupus
2022, Vol. 0(0) 1–11

DOI: 10.1177/09612033221102073
journals.sagepub.com/home/lup
SAGE

Immunogenicity, safety, and antiphospholipid antibodies after SARS-CoV-2 vaccine in patients with primary antiphospholipid syndrome

Flavio Signorelli[1,2], Gustavo Guimarães Moreira Balbi[1,3], Nadia E Aikawa[4], Clovis A Silva[4], Léonard de Vinci Kanda Kupa[1], Ana C Medeiros-Ribeiro[1], Emily FN Yuki[1], Sandra G Pasoto[1], Carla GS Saad[1], Eduardo F Borba[1], Luciana Parente Costa Seguro[1], Tatiana Pedrosa[1], Vitor Antonio de Angeli Oliveira[1], Ana Luisa Cerqueira de Sant'Ana Costa[1], Carolina T Ribeiro[1], Roseli Eliana Beseggio Santos[5], Danieli Castro Oliveira Andrade[1,†] and Eloisa Bonfá[1,†]

Abstract

Objective: Coronavirus disease 19 (COVID-19) has an increased risk of coagulopathy with high frequency of antiphospholipid antibodies (aPL). Recent reports of thrombosis associated with adenovirus-based vaccines raised concern that SARS-CoV-2 immunization in primary antiphospholipid syndrome (PAPS) patients may trigger clotting complications. Our objectives were to assess immunogenicity, safety, and aPL production in PAPS patients, after vaccinating with Sinovac-CoronaVac, an inactivated virus vaccine against COVID-19.

Methods: This prospective controlled phase-4 study of PAPS patients and a control group (CG) consisted of a two-dose Sinovac-CoronaVac (D0/D28) and blood collection before vaccination (D0), at D28 and 6 weeks after second dose (D69) for immunogenicity/aPL levels. Outcomes were seroconversion (SC) rates of anti-SARS-CoV-2 S1/S2 IgG and/or neutralizing antibodies (NAb) at D28/D69 in naïve participants. Safety and aPL production were also assessed.

Results: We included 44 PAPS patients (31 naïve) and 132 CG (108 naïve) with comparable age (p=0.982) and sex (p>0.999). At D69, both groups had high and comparable SC (83.9% vs. 93.5%, p=0.092), as well as NAb positivity (77.4% vs. 78.7%, p=0.440), and NAb-activity (64.3% vs. 60.9%, p=0.689). Thrombotic events up to 6 months or other moderate/severe side effects were not observed. PAPS patients remained with stable aPL levels throughout the study at D0 vs. D28 vs. D69: anticardiolipin (aCL) IgG (p=0.058) and IgM (p=0.091); anti-beta-2 glycoprotein I (aβ2GPI) IgG (p=0.513) and IgM (p=0.468).

Conclusion: We provided novel evidence that Sinovac-CoronaVac has high immunogenicity and safety profile in PAPS. Furthermore, Sinovac-CoronaVac did not trigger thrombosis nor induced changes in aPL production.

Keywords

COVID-19, vaccine immunogenicity, SARS-CoV-2 vaccine, antiphospholipid syndrome, antiphospholipid antibodies

[1]Rheumatology Division, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, Brazil
[2]Rheumatology Division, Hospital Universitário Pedro Ernesto, Universidade do Estado do Rio de Janeiro, Brazil
[3]Rheumatology Division, Hospital Universitário, Universidade Federal de Juiz de Fora, Brazil
[4]Pediatric Rheumatology Unit, Instituto da Criança, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, Brazil
[5]Central Laboratory Division, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, Brazil

[†]DA and EB contributed equally to this manuscript.

Corresponding author:
Eloisa Bonfá, Sala 3190, Av. Dr Arnaldo, 455, 3° andar, São Paulo, SP 01246-903, Brazil.
Email: eloisa.bonfa@hc.fm.usp.br

Background

Coronavirus disease 19 (COVID-19) has an increased risk of coagulopathy, especially the occurrence of thromboembolic events. The intense inflammatory response evoked by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication may induce a dysregulation of coagulation toward a hypercoagulable state,[1-3] and both large vessels and microcirculation may be affected.[4-6]

Antiphospholipid syndrome (APS) is the most frequent acquired thrombophilia.[7] Half of the cases, known as primary APS (PAPS), occur without the concomitance of other autoimmune rheumatic diseases (ARD).[8] APS is characterized by the persistent presence of antiphospholipid antibodies (aPL), namely, lupus anticoagulant (LA), IgG, and/or IgM aCL and IgG and/or IgM aβ2GPI, which play an important role in the pathogenesis of thrombosis in those patients.[9]

Interestingly, both diseases share common mechanisms of thrombosis: activation of endothelial cells, resulting in inhibition of endothelial nitric oxide synthase production, and consequently, decreasing nitric oxide production; complement activation; and unchecked inflammatory signals responsible for the formation of neutrophil extracellular traps (NETosis).[10]

Recent studies reported the presence of aPL in patients infected with SARS-CoV-2.[11-14] Furthermore, infectious etiologies may act as the "second hit," crucial for the thrombogenesis in APS and/or aPL-positive patients.[15-16] Therefore, vaccinating these patients to prevent COVID-19 is of utmost importance.

Paradoxically, two of the vaccines against SARS-CoV-2 using adenovirus platforms developed by AstraZeneca and Janssen have been associated with the occurrence of rare and atypical thromboembolic events, especially in women under 50 years of age, a condition that has been called vaccine-induced immune thrombotic thrombocytopenia (VITT).[17,18] As a consequence, vaccinating patients with thrombophilia using other platforms, such as inactivated virus or mRNA, may be preferable in this subset of patients. However, studies on the efficacy and safety of those vaccines in APS are still lacking.

CoronaVac (Sinovac Life Sciences, Beijing, China) is an inactivated vaccine against COVID-19, which is supporting vaccination campaigns in more than 40 countries, including Brazil, and has shown good tolerance and efficacy in inducing humoral responses against SARS-CoV-2 in the general population.[19-21] Jara et al.[22] demonstrated that Sinovac-CoronaVac reduced rates of infection, hospitalization, ICU admission, and death by 65.9%, 87.5%, 90.3%, and 86.3%, respectively, in the overall population of 10.2 million people in Chile.

The aims of the present prospective study were to evaluate immunogenicity of Sinovac-CoronaVac vaccine in naïve PAPS patients compared to a balanced age- and sex-control group (CG). We further assessed safety, including thrombotic events, and the possible vaccine-induced aPL production throughout the study period.

Methods

Study design

This study is a subgroup analysis of patients with PAPS from a large phase four prospective controlled trial with ARD patients performed at a single tertiary center in Brazil.[23]

Patients and controls

All consecutive PAPS patients who fulfilled the current classification criteria for PAPS (Sidney) [9] and were regularly followed in our Outpatient Rheumatology Clinics and were ≥18 years old were invited to participate. Subsequently, a CG of hospital maintenance, administrative personal, or their relatives balanced by sex and age (±5 years differences) using an Excel program (ratio 1PAPS: 3CG) were also invited to participate. Exclusion criteria for both groups were the following: ARD (other than APS, for the patient's group), use of immunosuppressive drugs, HIV infection, history of anaphylactic response to vaccine components, acute febrile illness or symptoms compatible to COVID-19 at vaccination, previous demyelinating disease (including Guillain-Barré syndrome), symptomatic heart failure (class III or IV), previous vaccination with any SARS-CoV-2 vaccine, history of vaccination with live virus vaccine in the previous 4 weeks or with virus vaccine inactivated in the previous 2 weeks, history of having received blood products in the previous 6 months, individuals who refused to participate in the study, and hospitalized patients.

Participants who developed RT-PCR-confirmed COVID-19 after receiving the first vaccine dose (incident cases) and with positive COVID-19 serology and/or NAb at baseline (collected on the day of vaccination) were excluded from the immunogenicity and aPL analysis; however, they were included in the safety evaluation.

Vaccine protocol

PAPS patients and CG were scheduled to receive a two-dose vaccine. The first dose was given on February 9–18th 2021 (D0, with baseline blood collection immediately before it); the second dose was given 28 days later (D28, with blood collection immediately before it). A third blood sample was obtained 6 weeks after the second dose at day 69 (D69). This protocol was delayed 4 weeks for participants with incident COVID-19 infection during the study. Ready-to-use

syringes loaded with CoronaVac (Sinovac Life Sciences, Beijing, China, batch #20200412), that consists of 3 μg in 0.5 mL of β-propiolactone inactivated SARS-CoV-2 (derived from the CN02 strain of SARS-CoV-2 grown in African green monkey kidney cells—Vero 25 cells) with aluminum hydroxide as an adjuvant, were administered intramuscularly in the deltoid area. The sera of each blood sample (20 mL) from all participants obtained at days D0, D28, and D69 were stored in a −70°C freezer.

Anti-SARS-CoV-2 S1/S2 IgG antibodies

A chemiluminescent immunoassay was used to measure human IgG antibodies against the S1 and S2 proteins in the receptor binding domain (RBD) (Indirect ELISA, LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy). Seroconversion rate (SC) was defined as positive serology (>15.0 UA/mL) post vaccination, since only patients with pre-vaccination negative serology were included. Geometric mean titers (GMT) of these antibodies and 95% confidence intervals were also calculated at all time points, attributing the value of 1.9 UA/mL (half of the lower limit of quantification 3.8 UA/mL) to undetectable levels (<3.8 UA/mL). The factor increase in GMT (FI-GMT) is the ratio of the GMT after vaccination to the GMT before vaccination, used to demonstrate growth in IgG titers. They are also presented and compared as geometric means and 95% confidence intervals (CI).

SARS-CoV-2 cPass virus-neutralization antibodies

The SARS-CoV-2 sVNT Kit (GenScript, Piscataway, NJ, USA) was performed according to manufacturer instructions. This analysis detects circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the RBD of the viral spike glycoprotein with the ACE2 cell surface receptor. The tests were performed on the ETI-MAX-3000 equipment (DiaSorin, Italy). The samples were classified as either "positive" (inhibition ≥30%) or "negative" (inhibition <30%), as suggested by the manufacturer.[24] The frequency of positive samples was calculated at all time points. Medians (interquartile range) of the percentage of neutralizing activity only for positive samples were calculated.

Outcomes

Immunogenicity outcome was assessed by two criteria SC rates of total anti-SARS-Cov-2 S1/S2 IgG and presence of NAb at D69. Other endpoints were the following: anti-S1/S2 IgG SC and presence of NAb at D28 (after vaccine first dose); geometric mean titers of anti-S1/S2 IgG and their FI-GMT at D28 and D69; and median (interquartile range) neutralizing activity of NAb at D28 and D69.

Vaccine adverse events and incident cases of COVID-19

Patients and CG were advised to report any side effects of the vaccine. They received on D0 (first dose) and on D28 (second dose) a standardized diary for local and systemic manifestations. The standardized diary of adverse events (AE) was carefully reviewed with each participant on the day of the second dose (D28) and at the last visit (D69). COVID-19 incident cases were followed for 40 days (from D0 to 10 days after the second dose [D39]) and thereafter for the following 40 days (from D40 to D79).

Vaccine AE severity was defined according to WHO definitions.[25] A rigorous surveillance for any kind of thrombotic event was performed during a period of 6 months after full-vaccination.

Additionally, all participants were instructed to communicate any manifestation associated or not with COVID-19 through telephone, smartphone instant messaging, or email. Suspicious cases of COVID-19 were instructed to seek medical care near the residence and, if recommended, to come to our tertiary hospital to have the RT-PCR exam or in-person visit. Patients were clinically followed for 6 months (August 18, 2021).

Study data were collected and managed using REDCap electronic data capture tools hosted at our Institution.[26-27]

RT-PCR for SARS-CoV-2

Clinical samples for SARS-CoV-2 RT-PCR consisted of nasopharyngeal and oropharyngeal swabs, using a laboratory developed test.[28]

Antiphospholipid antibodies

We assessed the criteria antiphospholipid antibodies IgG/IgM aCL and IgG/IgM anti-β2GPI in PAPS patients. Peripheral blood samples were collected in dry tubes (2 tubes), respecting the time between collection and centrifugation of at most 1 hour. Samples were centrifuged at 3200 r/min for 15 min and aliquoted in a volume of 500 μL. The aCL antibodies were detected by commercial fluoro immunoenzymatic assay (EliA) Thermo Scientific™/Phadia™ 250 Immunoassay Analyzers and they were considered positive if present in medium or high titers (≥40 GPL or MPL). The aβ2GPI antibodies were measured through the enzyme-linked immunosorbent assay (ELISA) QUANTALite®, InovaDiagnostics and their positivity was defined if titers were > 20UI/mL. Antiphospholipid antibodies at D28 and D69 were compared to baseline (D0) to verify if there was any increase in titers after vaccination. The thrombosis score risk aGAPSS (adjusted Global AntiPhospholipid Syndrome Score) that includes the three criteria aPL,[29-30] besides arterial hypertension and

dyslipidemia, was calculated at baseline and at D69 using LA previously registered in our electronic database. LA detection was performed according to updated guidelines.[31]

Statistical analysis

A convenience sample of PAPS patients was selected with a CG in a 1:3 ratio. Continuous variables are presented as medians (interquartile ranges) with intergroup comparison using Mann–Whitney test. Categorical variables are presented as number (percentage) and compared using chi-square or Fisher's exact tests, as appropriate. Continuous data regarding anti-S1/S2 serology titers are presented as geometric means (95% CI) and compared with the same tests, but in Napierian logarithm (ln) transformed data. Longitudinal comparisons of ln-transformed anti-S1/S2 IgG titers between PAPS and CG were performed using generalized estimating equations (GEE) with normal marginal distribution and gamma distribution, respectively. Results were followed by Bonferroni multiple comparisons to identify differences between groups and time points. Multivariate logistic regression analyses were performed using as dependent variables SC or presence of NAb, and as independent variables those with p <0.2 in univariate analysis. The isotypes of each aPL were analyzed categorically (according to aPL cutoff positivity definitions) using Chi-square test and continuously by Friedman Repeated Measures Analysis of Variance on Ranks at D0, D28, and D69. aGAPSS score of APS patients was also compared between the three time points using Friedman Repeated Measures Analysis of Variance on Ranks.

Statistical significance was defined as p <0.05. All statistical analyses were performed using IBM-SPSS for Windows software version 22.0.

Ethics statement

The protocol was approved by the National and Institutional Ethical Committee of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), Brazil (CAAE: 42566621.0.0000.0068). It was in accordance with the Declaration of Helsinki and local regulations, and all participants signed a written informed consent before enrollment.

Results

Participants

We initially selected 63 patients, but six patients did not attend the vaccine appointment, one patient had symptoms compatible with COVID-19 at the day of vaccination and 12 patients had associated systemic lupus erythematosus (SLE) and were excluded. The remaining 44 PAPS patients and 132 controls were included in the study. Forty-three patients had thrombotic criteria (97.7%) and 18 (40.9%) had obstetric criteria. Only one patient was classified as exclusively obstetric. Triple positivity was present in 45.4% of cases (Table 1). The number of triple positives was even higher (54.8%) considering only the 31 naïve-PAPS.

PAPS patients and CG had comparable median ages (46 [31–73] vs. 46 [31–78] years, p=0.982) and female sex (86.4% in both groups, p=1.0) at study entry. The mean duration of disease in PAPS patients was 16.7 ± 8.4 years. Of note, the PAPS group had more stroke than CG (29.5% vs. 0%, p<0.001), besides dyslipidemia (59.1% vs. 8.3%, p<0.001) and smoking (38.6% vs. 8.3%, p<0.001). These characteristics are shown in Table 1.

Vaccine immunogenicity

For this analysis, we excluded 37 (21.0%) participants (13 PAPS patients and 24 CG) due to pre-vaccination positive COVID-19 serology (6 PAPS and 18 CG) and/or NAb (1 PAPS and 3 CG) and the incidents confirmed cases of COVID-19 during the study (1 PAPS and 3 CG). Further exclusions for the immunogenicity analyses were related to continuous immunosuppression (not related to APS): two patients were using azathioprine and prednisone (one due to autoimmune hepatitis and the other due to idiopathic interstitial pulmonary disease); one patient with renal transplant was on mycophenolate mofetil, tacrolimus, and prednisone; one patient with cardiac transplant was on mycophenolate mofetil and cyclosporine; and one patient was using prednisone to treat livedoid vasculopathy.

The final immunogenicity analysis included 31 naïve-PAPS patients and 108 controls. Flow chart of the study is illustrated in Figure 1.

Anti-SARS-CoV-2 IgG antibodies

There was a modest initial response of anti-SARS-CoV-2 IgG in both groups after the first dose with comparable SC in naïve-PAPS patients a CG at D28 (25.8% vs. 30.6%, p=0.609). The SC rates at D69 increased approximately 3-fold after the second dose with similar immunogenicity for naïve-PAPS and GC groups: SC rates (83.9% vs. 93.5%, p=0.092) and geometric mean titers (GMT) (50.2 [95%CI 34.5–73.2] in PAPS vs. 61.7 [95%CI 52.8–72.3] in CG, p=0.249). The factor increase in GMT (FI-GMT) at D69 was also elevated in naïve-PAPS and CG (21.4 [95%CI 14.5–31.6] vs. 26.5 [95%CI 22.3–31.4], p=0.586) and at D69, respectively (Table 2).

According to Bonferroni's multiple comparison, there was a significant GMT increase when we performed longitudinal comparisons of GMT in naïve-PAPS patients at baseline versus D28 and D69 (p<0.001, for both) and at D28 vs. D69 (p<0.001). Likewise, the results of longitudinal

GMT comparisons in CG at D28 and D69 vs. baseline and between D69 vs. D28 also showed a significant increase (p<0.001, for all comparisons) (Table 2).

SARS-CoV-2 cPass virus-neutralization antibodies (NAb)

The frequency of NAb at D28 was lower in naïve-PAPS patients than CG (16.1% vs. 35.2%, p=0.043), with a robust rise at D69 and comparable NAb positivity rates among both groups (77.4% vs. 78.7%, p=0.440). NAb-activity was comparable in naïve-PAPS patients and CG at D28 (38.1% [32.0–55.5] vs. 43.7% [34.2–66.4], p=0.275) and D69 (64.3 [49.0–77.0%] vs. 60.9 [45.6–81.3%], p=0.689) (Table 3).

Antiphospholipid antibodies and vaccination

High titers of aCL at baseline were identified in 13/31 (41.9%) of the naïve-APS patients (seven of IgG isotype, four of IgM isotype, and 1 with both isotypes). Fourteen (45.2%) patients had high titers of aβ2GPI at baseline (four with IgG isotype, eight of IgM isotype, and two with both isotypes). All patients remained positive for aCL and/or aβ2GPI without significant changes in titers, but one patient with negative IgM aCL (5 MPL) and IgM aβ2GPI (5 UI/mL) at baseline and at D28 (IgM aCL: four MPL and IgM aβ2GPI:4 UI/mL) had an increment to 48 MPL and 42 UI/mL, respectively, at day 69.

No significant difference was found between samples collected before and after vaccination for all four autoantibodies (Figure 2). In the quantitative analysis, titers remained stable over time. In the qualitative assessment, frequencies of positivity also did not change for all aPL: IgG aCL positivity rates were 25.8% (n=8/31) vs. 25.8% (n=8/31) vs. 22.6% (n=7/31), p=0.944, at D0, D28, and D69; IgM aCL positivity rates were 16.1% (n=5/31) vs. 16.1% (n=5/31) vs. 19.4% (n=6/31), p=0.927, at D0, D28, and D69; IgG aβ2GPI positivity rates were 12.9% (n=4/31) vs. 12.9% (n=4/31) vs. 16.1% (n=5/31), p=0.914, at D0, D28, and D69; and IgM aβ2GPI positivity rates were 16.1% (n=5/31) vs. 16.1% (n=5/31) vs. 19.4% (n=6/31), p=0.927, at D0, D28, and D69.

The median (interquartile range) aGAPSS of the 31 naïve-APS patients did not modify after completing vaccination (D0 vs D28 vs D69: 13 [4–17] vs. 13 [4–17] vs. 13 [4–17], p=0.717).

Vaccine safety and tolerance

We did not observe any moderate/severe AE in any group. Local and systemic reactions were more common in the PAPS group after the first dose compared to controls, but not after the second dose. The overall description of AE in PAPS patients and controls is summarized in Table 4.

Table 1. Baseline characteristics of primary antiphospholipid syndrome patients and controls.

	PAPS (n=44)	Controls (n=132)	p-Value
Demographics			
Current age, years	46 (31–73)	46 (31–78)	0.982
Age at diagnosis, years	29 (17–67)	-	-
Disease duration, years	16.7 ± 8.4	-	-
Female sex	44 (86.4)	114 (86.4)	>0.999
Caucasian race	27 (61.4)	64 (48.5)	0.139
Comorbidities			
Systemic arterial hypertension	18 (40.9)	39 (29.5)	0.163
Diabetes mellitus	3 (6.8)	16 (12.1)	0.411
Dyslipidemia	26 (59.1)	11 (8.3)	<0.001
Obesity	21 (47.7)	42 (32.3)	0.066
Current smoking	17 (38.6)	11 (8.3)	<0.001
APS criteria manifestations			
Thrombotic	43 (97.7)	-	-
Arterial	21 (47.7)	-	-
Stroke	13 (29.5)	0 (0)	<0.001
Venous	25 (56.8)	-	-
Obstetric	18 (40.9)	-	-
aPL profile			
Single positivity	11 (25.0)	-	-
Double positivity	13 (29.5)	-	-
Triple positivity	20 (45.5)		
APS treatment			
VKA	39 (88.6)	-	-
LMWH	3 (6.8)	-	-
LDA	8 (18.2)	-	
Hydroxychloroquine	17 (38.6)	-	

Results are expressed in mean ± standard deviation, median (minimum and maximum values), and n (%).
PAPS—primary antiphospholipid syndrome; aPL—antiphospholipid antibody; VKA—vitamin K antagonist; LMWH—low-molecular-weight heparin; LDA—low dose aspirin.

COVID-19 incident cases

During the study, four participants (one PAPS patient and three CG) had incident symptomatic cases of COVID-19, all confirmed by RT-PCR. All cases occurred from D0 to D32 and none of them was hospitalized.

Discussion

To the best of our knowledge, this is the first study to demonstrate that the Sinovac-CoronaVac vaccine is highly immunogenic and safe in PAPS patients and did not trigger short- and medium-term thrombosis or increase of aPL-related antibodies production.

Recent studies focusing on an overall evaluation of mRNA COVID-19 immunized ARD patients have shown a

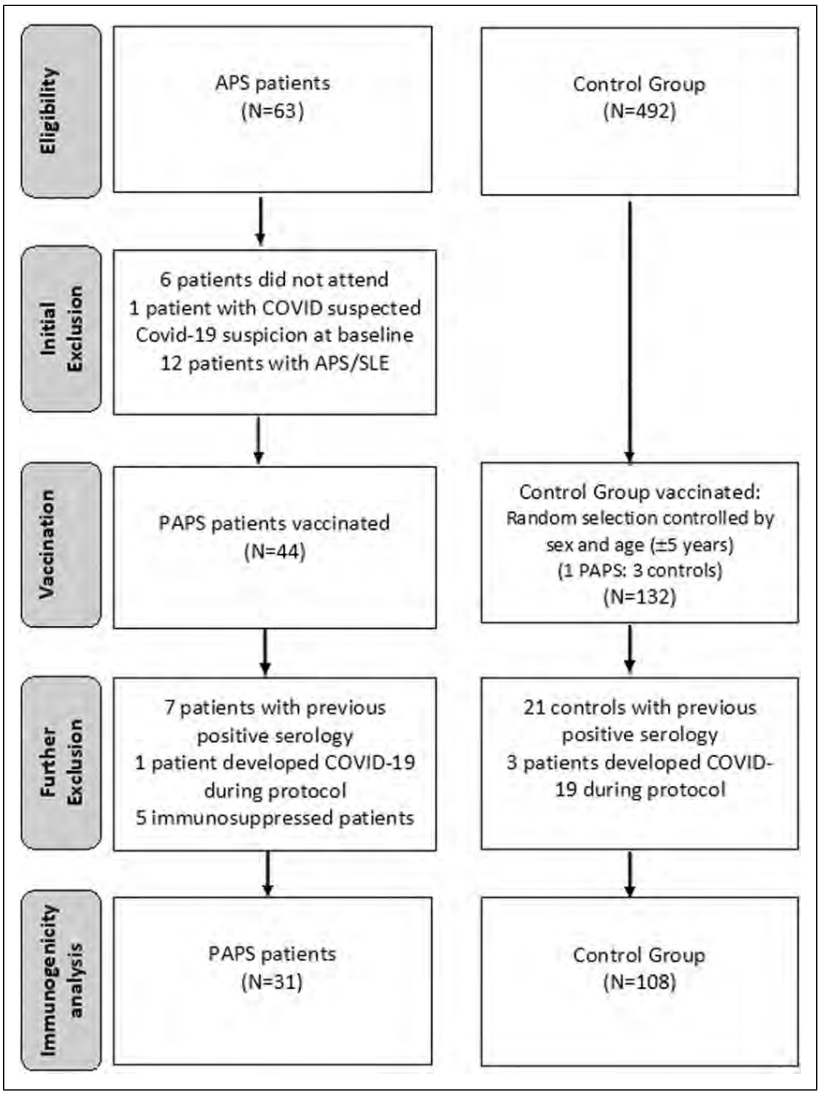


Figure 1. Flowchart of patients and controls submitted to Sinovac-CoronaVac vaccination.

Table 2. Seroconversion rates and anti-SARS-CoV-2 S1/S2 IgG titers before and after CoronaVac in näive-PAPS and controls.

	Seroconversion (SC)		Geometric mean titer (GMT)			Factor increase in GMT	
	D28	D69	D0	D28	D69	D28	D69
PAPS, *n*=31	8 (25.8)	26 (83.9)	2.4 (2.0–2.7)	7.7 (5.1–11.6)[a]	50.2 (34.5–73.2)[a,b]	3.3 (2.2–4.9)	21.4 (14.5–31.6)
Controls, *n*=108	33 (30.6)	101 (93.5)	2.3 (2.1–2.6)	9.8 (7.6–12.6)[c]	61.7 (52.8–72.3)[c,d]	4.2 (3.4–5.1)	26.5 (22.3–31.4)
p-Value (PAPS vs CG)	0.609	0.092	0.936	0.359	0.249	0.600	0.586

PAPS—Primary antiphospholipid syndrome; CG—control group; SC—Seroconversion (defined as post-vaccination titer >15 AU/mL—Indirect ELISA, LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy); GMT—Geometric mean titers (AU/mL).
Frequencies of SC are presented as number (%) and they were compared using chi-square between PAPS patients and CG at pre-specified time points (D28 and D69). IgG antibody titers and FI-GMT are expressed as geometric means with 95% confidence interval (95%CI). Comparisons of ln-transformed anti-S1/S2 IgG titers between PAPS and CG were performed using generalized estimating equations (GEE) with normal marginal distribution and gamma distribution, respectively. Results were followed by Bonferroni multiple comparisons to identify differences between groups and time points.
[a]p<0.001 for longitudinal comparisons of GMT in PAPS patients at D28 and D69 vs. baseline.
[b]p<0.001 for longitudinal comparison of GMT in PAPS patients at D69 vs. D28.
[c]p<0.001 for longitudinal comparison of GMT in control at D28 and D69 vs. baseline.
[d]p<0.001 for longitudinal comparison of GMT in control at D69 vs. D28.

Table 3. Frequency of neutralizing antibodies and neutralizing activity (%) after CoronaVac in näive-PAPS compared to controls.

	After vaccine 1st dose		After vaccine 2nd dose	
	Subjects with positive NAb, n (%)	Neutralizing activity (%) median (interquartile range)	Subjects with positive NAb, n (%)	Neutralizing activity (%) median (interquartile range)
PAPS, *n*=31	5 (16.1)[a]	38.1 (32–55.5)	24 (77.4)	64.3 (49.0–77.0)
Controls, *n*=108	38 (35.2)	43.7 (34.2–66.4)	85 (78.7)	60.9 (45.6–81.3)
p-Value (PAPS vs CG)	*p* =0.043	*p*=0.275	*p*=0.440	*p*=0.689

Results are expressed in median (interquartile range) and n (%).
Nab—neutralizing antibodies; PAPS—primary antiphospholipid syndrome; CG—control group.
Positivity for Nab defined as a neutralizing activity ≥30% (cPass sVNT Kit, GenScript, Piscataway, USA).
[a]p <0.05 in comparison to controls.

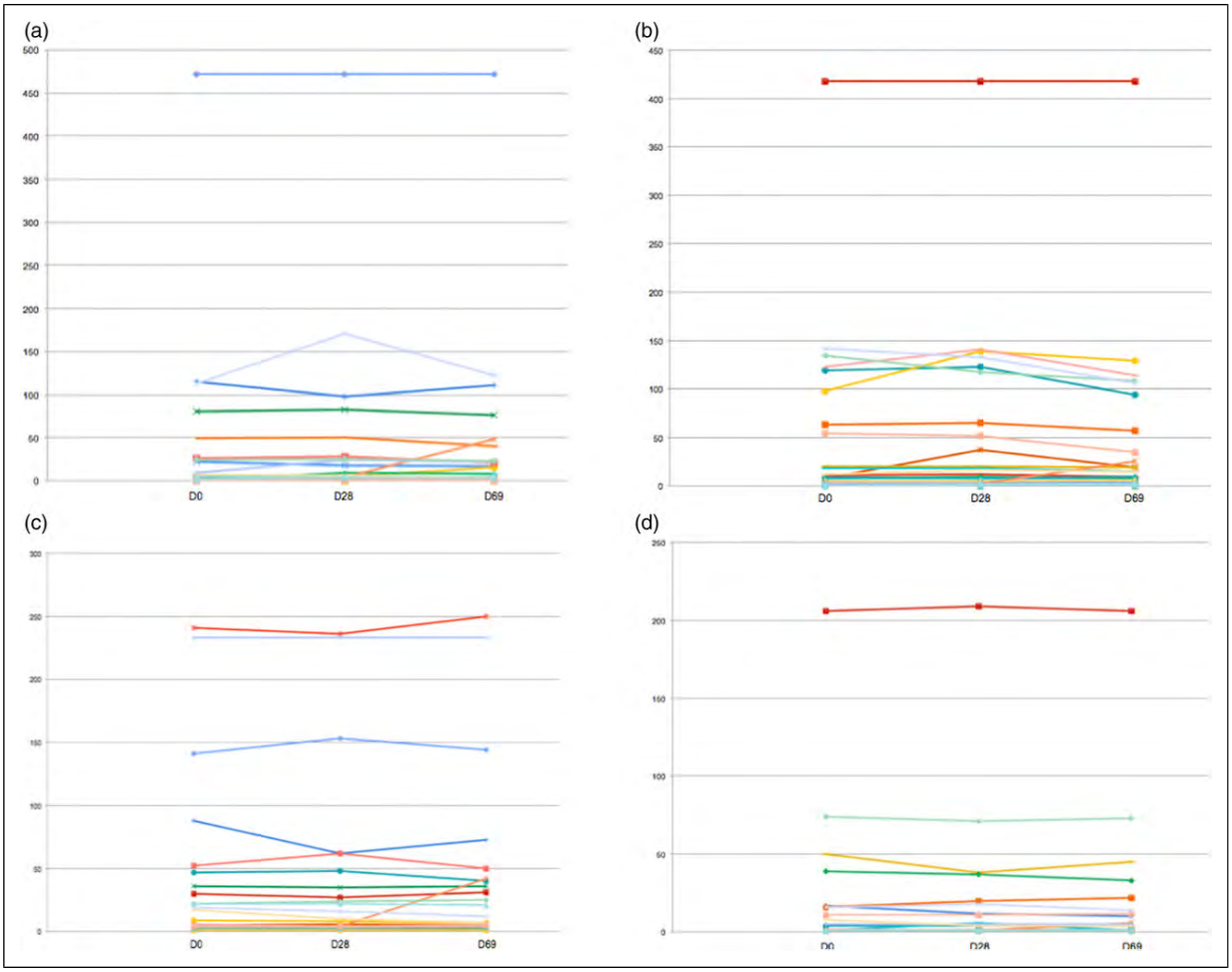


Figure 2. Antiphospholipid antibody titers evaluation in näive primary antiphospholipid patients before (baseline—D0) and after Sinovac-CoronaVac vaccination (first dose—D28 and second dose—D69). (a) Anticardiolipin antibody IgM (aCL, titers in MPL), (b) anticardiolipin antibody IgG (aCL, titers in GPL), (c) anti-beta-2 glycoprotein I IgM (aβ2GPI, titers in UI/mL), and (d) anti-beta-2 glycoprotein I IgG (aβ2GPI, titers in UI/mL).

good safety profile, with no severe AE or underlying disease flare.[30,31] However, lower antibody titers compared to controls were observed, which may impact protection against the virus.[32-33] In line with these findings, our recent study revealed a moderate, but reduced SC rate with Sinovac-CoronaVac in 910 adults with naïve ARD (vs. 182 naïve volunteers in CG). Immunosuppressive drugs and prednisone were identified as factors associated with

Table 4. Adverse events of CoronaVac vaccination in primary antiphospholipid syndrome patients and controls.

	After vaccine 1st dose			After vaccine 2nd dose		
	PAPS (*n*=44)	Controls (*n*=132)	*p*-Value	PAPS (*n*=44)	Controls (*n*=132)	*p*-Value
No symptoms	26 (59.1)	81 (61.4)	0.789	25 (59.5)	86 (66.7)	0.400
Local reactions (at the injection site)	14 (31.8)	27 (20.5)	0.123	8 (19.0)	26 (20.2)	0.876
Pain	12 (27.3)	22 (16.7)	0.123	6 (14.3)	25 (19.4)	0.457
Erythema	5 (11.4)	1 (0.8)	0.004	2 (4.8)	1 (0.8)	0.150
Swelling	2 (4.5)	5 (3.8)	>0.999	3 (7.1)	8 (6.2)	0.732
Bruise	6 (13.6)	4 (3.0)	0.017	0 (0)	1 (0.8)	>0.999
Pruritus	2 (4.5)	1 (0.8)	0.155	2 (4.8)	6 (4.7)	>0.999
Induration	4 (9.1)	6 (4.5)	0.270	2 (4.8)	9 (7.0)	>0.999
Systemic reactions	16 (36.4)	39 (29.5)	0.398	15 (35.7)	37 (28.7)	0.390
Fever	2 (4.5)	2 (1.5)	0.260	2 (4.8)	3 (2.3)	0.597
Malaise	6 (13.6)	5 (3.8)	0.019	3 (7.1)	13 (10.1)	0.764
Somnolence	6 (13.6)	10 (7.6)	0.226	1 (2.4)	9 (7.0)	0.454
Lack of appetite	2 (4.5)	4 (3.0)	0.641	1 (2.4)	6 (4.7)	>0.999
Nausea	6 (13.6)	1 (0.8)	0.001	3 (7.1)	9 (7.0)	>0.999
Vomit	1 (2.3)	1 (0.8)	0.439	0 (0)	1 (0.8)	>0.999
Diarrhea	4 (9.1)	7 (5.3)	0.471	3 (7.1)	7 (5.4)	0.709
Abdominal pain	3 (6.8)	6 (4.5)	0.693	3 (7.1)	7 (5.4)	0.709
Vertigo	5 (11.4)	3 (2.3)	0.024	2 (4.8)	6 (4.7)	>0.999
Tremor	3 (6.8)	0 (0)	0.015	1 (2.4)	2 (1.6)	0.573
Headache	6 (13.6)	16 (12.1)	0.792	8 (19.0)	23 (17.8)	0.859
Fatigue	8 (18.2)	5 (3.8)	0.002	5 (11.9)	14 (10.9)	0.851
Sweating	4 (9.1)	2 (1.5)	0.035	3 (7.1)	3 (2.3)	0.159
Myalgia	4 (9.1)	3 (2.3)	0.067	6 (14.3)	14 (10.9)	0.548
Muscle weakness	5 (11.4)	4 (3.0)	0.044	5 (11.9)	10 (7.8)	0.409
Arthralgia	6 (13.6)	5 (3.8)	0.019	4 (9.5)	9 (7.0)	0.737
Back pain	7 (15.9)	7 (5.3)	0.024	3 (7.1)	16 (12.4)	0.413
Cough	4 (9.1)	4 (3.0)	0.109	0 (0)	6 (4.7)	0.338
Sneezing	4 (9.1)	6 (4.5)	0.270	5 (11.9)	11 (8.5)	0.514
Coryza	3 (6.8)	11 (8.3)	>0.999	5 (11.9)	16 (12.4)	0.932
Stuffy nose	6 (13.6)	6 (4.5)	0.038	2 (4.8)	12 (9.3)	0.522
Sore throat	0 (0)	5 (3.8)	0.333	2 (4.8)	7 (5.4)	>0.999
Shortness of breath	2 (4.5)	4 (3.0)	0.641	0 (0)	4 (3.1)	0.573
Conjunctivitis	0 (0)	0 (0)	-	0 (0)	1 (0.8)	>0.999
Pruritus	2 (4.5)	0 (0)	0.061	2 (4.8)	2 (1.6)	0.253
Skin rash	1 (2.3)	2 (1.5)	>0.999	0 (0)	1 (0.8)	>0.999

Results are presented in n (%). PAPS—primary antiphospholipid syndrome.

diminished immunogenicity evaluating the entire group of ARD patients.[23]

However, PAPS patients may have some distinct clinical and immunological features [34] compared to other ARDs. A previous study published by our group evaluating the response to the H1N1 vaccine in 1668 ARD patients demonstrated that PAPS patients presented higher rates of SC than several other ARDs.[35] The present study with Sinovac-CoronaVac vaccine showed that PAPS patients had a high SC and high NAb positivity, comparable to the CG. The most likely explanation is the fact that the cornerstone of treatment in this syndrome is lifelong anticoagulation and not immunosuppressive therapy.[36] The accuracy of this data was improved by the fact that both groups were balanced by age and sex, one of the most important parameters to influence vaccine response.[37] In addition, the impact of previous exposure in vaccine response was excluded, since only naïve-PAPS patients were evaluated for immunogenicity. In fact, previous studies have demonstrated that vaccine-induced antibody response is greatly enhanced in pre-exposed individuals.[38-39]

The safety profile of inactivated COVID-19 vaccines has been tested and confirmed by mass immunization programs; those vaccines are highly relevant for the population

evaluated in the present study.[40] Our PAPS patients had more minor adverse effects compared to controls. Perhaps the awareness of having a thrombophilia might have alerted them to report any symptom after the first dose. The occurrence of more bruises was expected because of anticoagulation.

Even though the thrombotic risk assessed with aGAPSS in our PAPS patients was very high, no thrombotic event was recorded during our study.[41] In addition, these patients have a high frequency of comorbidities associated with endothelial dysfunction, such as hypertension, obesity, and dyslipidemia, which may also favor clot events.[42-44] Despite the very small sample size, it is reassuring that no cases of venous and arterial thromboses were observed in this high-risk population, after 6 months of follow-up.

Supporting this notion, aPL titers were comparable before and after complete vaccination, an encouraging finding since aPL has an important role in the PAPS thrombogenesis.[10] Consistent with this observation, we have not detected a significant production of aPL-related antibodies nor thrombotic events after the pandemic influenza immunization in PAPS patients.[45] Furthermore, a larger Chinese study with 406 healthy-workers immunized with inactivated SARS-CoV-2 vaccine (BBIBPCorV, Sinopharm, Beijing, China) also found no significant difference in aPL measurement in serial blood samples before and 4 weeks after the second dose.[46]

Our study has some limitations. The routine blood collection used to perform immunogenicity assays of SARS-CoV-2 could not be extrapolated to LA functional assays, a known high-risk parameter for thrombosis in PAPS and perhaps also for COVID-19 infection.[47] Another flaw in our study was the small convenience sample size but very much related to the general prevalence of this disease in the population, which is approximately 50 per 100,000 population,[48] with numbers being even lower when considering only PAPS.

In conclusion, Sinovac-CoronaVac vaccine was highly immunogenic, demonstrated a good safety profile, and did not trigger short- and medium-term thrombosis or production of aPL in naïve-PAPS patients. Our findings support the recommendation of SARS-CoV-2 vaccination for PAPS patients.

Acknowledgments

We thank the contribution of the Central Laboratory Division, Registry Division, Security Division, IT Division, Superintendency, Pharmacy Division, and Vaccination Center (CRIE) for their technical support. We also thank the volunteers for participating in the three in-person visits of the protocol, handling the biological material and those responsible for the follow-up of all participants.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (#2015/03756-4 to SGP, CAS, NEA and EB, #2019/17272-0 to LVKK); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq #304984/2020-5 to CAS, #305242/2019-9 to EB, #306879/2018-2 to EFB), B3 - Bolsa de Valores do Brasil to EB, and Instituto Butantan supplied the study product and had no other role in the trial.

Trial registration

ClinicalTrials.gov- NCT04754698 first registered on February 8th 2021. https://clinicaltrials.gov/ct2/show/NCT04754698?term=NCT04754698&draw=2&rank=1

ORCID iDs

Flavio Signorelli https://orcid.org/0000-0002-5565-1017
Gustavo Guimarães Moreira Balbi https://orcid.org/0000-0003-0235-8834
Nadia E Aikawa https://orcid.org/0000-0002-7585-4348
Clovis A Silva https://orcid.org/0000-0001-9250-6508
Sandra G Pasoto https://orcid.org/0000-0002-7343-6804
Eduardo F Borba https://orcid.org/0000-0001-6194-5129
Danieli Castro Oliveira Andrade https://orcid.org/0000-0002-0381-1808
Eloisa Bonfá https://orcid.org/0000-0002-0520-4681

References

1. Chan NC and Weitz JI. COVID-19 coagulopathy, thrombosis, and bleeding. *Blood* 2020; 136: 381–383.
2. Levi M and van der Poll T. Inflammation and coagulation. *Crit Care Med* 2010; 38(2 Suppl): S26–S34.
3. Gustine JN and Jones D. Immunopathology of hyperinflammation in COVID-19. *Am J Pathol* 2021; 191: 4–17.
4. Price LC, McCabe C, Garfield B, et al. Thrombosis and COVID-19 pneumonia: the clot thickens! *Eur Respir J* 2020; 56: 2001608.
5. Suh YJ, Hong H, Ohana M, et al. Pulmonary embolism and deep vein thrombosis in COVID-19: A systematic review and meta-analysis. *Radiology* 2021; 298: E70–E80.
6. Tan BK, Mainbourg S, Friggeri A, et al. Arterial and venous thromboembolism in COVID-19: a study-level meta-analysis. *Thorax* 2021; 76: 970–979.
7. Radin M, Cecchi I, Rubini E, et al. Treatment of antiphospholipid syndrome. *Clin Immunol* 2020; 221: 108597.
8. Cervera R. Lessons from the "Euro-Phospholipid" project. *Autoimmun Rev* 2008; 7: 174–178.

9. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4: 295–306.
10. Wang X, Krouzman E, Andrade DCO, et al. COVID-19 and antiphospholipid antibodies: A position statement and management guidance from AntiPhospholipid Syndrome Alliance for Clinical Trials and InternatiOnal Networking (APS ACTION). *Lupus* 2021; 31: 143–160.
11. Tung ML, Tan B, Cherian R, et al. Anti-phospholipid syndrome and COVID-19 thrombosis: connecting the dots. *Rheumatol Advances Practice* 2021; 5: rkaa081.
12. Kipshidze N, Dangas G, White CJ, et al. Viral coagulopathy in patients with COVID-19: Treatment and care. *Clin Applied Thrombosis/hemostasis : Official Journal Int Acad Clin Appl Thrombosis/Hemostasis* 2020; 26: 1076029620936776.
13. Zuo Y, Estes SK, Ali RA, et al. Prothrombotic autoantibodies in serum from patients hospitalized with COVID-19. *Sci Translational Medicine* 2020; 12: eabd3876.
14. Taha M and Samavati L. Antiphospholipid antibodies in COVID-19: a meta-analysis and systematic review. *RMD Open* 2021; 7: e001580.
15. Willis R, Harris E and Pierangeli S. Pathogenesis of the antiphospholipid syndrome. *Semin Thromb Hemost* 2012; 38: 305–321.
16. Giannakopoulos B and Krilis SA. The pathogenesis of the antiphospholipid syndrome. *New Engl J Med* 2013; 368: 1033–1044.
17. El Hasbani G, Taher AT, Jawad A, et al. COVID-19, Antiphospholipid Antibodies, and Catastrophic Antiphospholipid Syndrome: A Possible Association? *Clin Medicine Insights. Arthritis Musculoskeletal Disorders* 2020; 13: 1179544120978667.
18. Makris M, Pavord S, Lester W, et al. Vaccine-induced immune thrombocytopenia and thrombosis (VITT). *Res Practice Thrombosis Haemostasis* 2021; 5: e12529.
19. Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; 21: 181–192.
20. Mallapaty S. WHO approval of Chinese CoronaVac COVID vaccine will be crucial to curbing pandemic. *Nature* 2021; 594: 161–162.
21. Palacios R, Batista AP, Albuquerque CSN, et al. Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in Brazil: The PROFISCOV study. *SSRN Electron J Published* 2021; 21(1):853.
22. Jara A, Undurraga EA, González C, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. *New Engl J Med* 2021; 385: 875–884.
23. Medeiros-Ribeiro AC, Aikawa NE, Saad CGS, et al. Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial. *Nat Med* 2021; 27: 1744–1751.
24. Taylor SC, Hurst B, Charlton CL, et al. A new SARS-CoV-2 dual-purpose serology test: Highly accurate infection tracing and neutralizing antibody response detection. *J Clinical Microbiology* 2021; 59: e02438.
25. World Health Organization. *World alliance for patient safety: WHO draft guidelines for adverse event reporting and learning systems: from information to action*. World Health Organization, 2005.
26. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform* 2019; 95: 103208.
27. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; 42: 377–381.
28. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill: Eur Commun Dis Bull* 2020; 25: 2000045.
29. Sciascia S, Sanna G, Murru V, et al. GAPSS: the Global Anti-Phospholipid Syndrome Score. *Rheumatology* 2013; 52: 1397–1403.
30. Radin M, Schreiber K, Costanzo P, et al. The adjusted Global AntiphosPholipid Syndrome Score (aGAPSS) for risk stratification in young APS patients with acute myocardial infarction. *Int J Cardiol* 2017; 240: 72–77.
31. Pengo V, Tripodi A, Reber G, et al. Update of the guidelines for lupus anticoagulant detection. *J Thromb Haemost* 2009; 7: 1737–1740.
32. Geisen UM, Berner DK, Tran F, et al. Immunogenicity and safety of anti-SARS-CoV-2 mRNA vaccines in patients with chronic inflammatory conditions and immunosuppressive therapy in a monocentric cohort. *Ann Rheum Dis* 2021; 80: 1306–1311.
33. Simon D, Tascilar K, Fagni F, et al. SARS-CoV-2 vaccination responses in untreated, conventionally treated and anticytokine-treated patients with immune-mediated inflammatory diseases. *Ann Rheum Dis* 2021; 80: 1312–1316.
34. Cervera R, Piette J-C, Font J, et al. Antiphospholipid syndrome: Clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum* 2002; 46: 1019–1027.
35. Saad CGS, Borba EF, Aikawa NE, et al. Immunogenicity and safety of the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune rheumatic diseases. *Ann Rheum Dis* 2011; 70: 1068–1073.
36. Signorelli F, Balbi GGM, Domingues V, et al. New and upcoming treatments in antiphospholipid syndrome: A comprehensive review. *Pharmacol Res* 2018; 133: 108–120.
37. Sadarangani M, Abu Raya B, Conway JM, et al. Importance of COVID-19 vaccine efficacy in older age groups. *Vaccine* 2021; 39: 2020–2023.
38. Sadarangani M, Marchant A and Kollmann TR. Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. *Nat Rev Immunol* 2021; 21: 475–484.

39. Ebinger JE, Fert-Bober J, Printsev I, et al. Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2. *Nat Med* 2021; 27: 981–984.
40. He Q, Mao Q, Zhang J, et al. COVID-19 Vaccines: Current Understanding on Immunogenicity, Safety, and Further Considerations. *Front Immunol* 2021; 12: 669339.
41. Radin M, Sciascia S, Erkan D, et al. The adjusted global antiphospholipid syndrome score (aGAPSS) and the risk of recurrent thrombosis: Results from the APS ACTION cohort. *Semin Arthritis Rheum* 2019; 49: 464–468.
42. Lowe GDO. Common risk factors for both arterial and venous thrombosis. *Br J Haematol* 2008; 140: 488–495.
43. Greinacher A, Thiele T, Warkentin TE, et al. Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. *New Engl J Med* 2021; 384: 2092–2101.
44. Hunter PR. Thrombosis after covid-19 vaccination. *BMJ* 2021; 373: n9.
45. de Medeiros DM, Silva CA, Bueno C, et al. Pandemic influenza immunization in primary antiphospholipid syndrome (PAPS): a trigger to thrombosis and autoantibody production? *Lupus* 2014; 23: 1412–1416.
46. Liu T, Dai J, Yang Z, et al. Inactivated SARS-CoV-2 vaccine does not influence the profile of prothrombotic antibody nor increase the risk of thrombosis in a prospective Chinese cohort. *Sci Bull* 2021; 66: 2312–2319.
47. Devreese KMJ, Linskens EA, Benoit D, et al. Antiphospholipid antibodies in patients with COVID-19: A relevant observation? *J Thromb Haemost* 2020; 18: 2191–2201.
48. Duarte-García A, Pham MM, Crowson CS, et al. The epidemiology of antiphospholipid syndrome: A population-based study. *Arthritis Rheumatol* 2019; 71: 1545–1552.

4.2. CoronaVac é segura para pacientes com câncer, mostra estudo de Hong Kong

Um estudo realizado em Hong Kong com 74 mil pacientes com câncer ou com histórico da doença voltou a mostrar que a CoronaVac é uma vacina segura para esse público, apresentando o menor índice de reações adversas na comparação com vacinas de RNA mensageiro. O trabalho foi publicado na revista Journal of Hematology & Oncology e conduzido por pesquisadores da Faculdade de Medicina da Universidade de Hong Kong.

Foram selecionados 74.878 indivíduos que já foram diagnosticados com câncer, divididos em dois grupos: pacientes com câncer ativo (25.789 ou 34% do total) e pessoas com histórico de câncer (49.089 ou 66%). Os voluntários receberam duas doses de CoronaVac ou Pfizer e os resultados foram comparados aos de pacientes não imunizados.

Aqueles que tomaram CoronaVac tiveram a menor incidência de reações adversas registrada em todo o ensaio. Nos pacientes com câncer ativo, a incidência de eventos adversos diários foi 0,13 para 10 mil pessoas vacinadas com CoronaVac, e 0,31 naquelas que receberam vacina produzida com a tecnologia de RNA mensageiro. Entre os não vacinados, a incidência foi maior, de 1,02.

Já entre as pessoas com histórico de câncer, a ocorrência de efeitos adversos diários da CoronaVac foi de 0,42 para 10 mil pessoas, enquanto a da vacina de RNA mensageiro foi de 0,55. No grupo que não recebeu nenhum imunizante, a taxa foi de 0,93.

Os pesquisadores concluíram que nenhuma das vacinas aumenta a frequência de eventos adversos nos pacientes com câncer ou com histórico da doença – pelo contrário,

aqueles não vacinados correm mais risco de sofrer com esses eventos. No período do estudo, em setembro de 2021, a taxa geral de vacinação contra a Covid-19 em Hong Kong atingiu 58,8%, mas apenas 30,2% dos pacientes com câncer haviam se vacinado.

"Os resultados reforçam a segurança do imunizante em indivíduos com câncer, o que pode incentivar o aumento da cobertura vacinal nesse grupo mais vulnerável", apontam os cientistas.

Evento adverso de interesse especial

Durante a pandemia, a Organização Mundial da Saúde (OMS) definiu que, no caso das campanhas de imunização contra a Covid-19, um evento adverso de interesse especial (AESI, na sigla em inglês) é um evento específico clinicamente significativo associado a uma vacina, cujos efeitos precisam ser cuidadosamente monitorados e avaliados.

Segundo a OMS, condições comumente identificadas como AESI incluem síndrome de Guillain-Barré, encefalomielite aguda, anafilaxia, eventos graves potencialmente relacionados a novas vacinas e adjuvantes, assim como qualquer doença cujo aumento da gravidade está associado ao imunizante, como o câncer.

Publicado em: 19/5/2022

Artigo

Kang *et al. Journal of Hematology & Oncology* (2022) 15:66
https://doi.org/10.1186/s13045-022-01265-9

Journal of Hematology & Oncology

BRIEF REPORT

Open Access

Safety of two-dose COVID-19 vaccination (BNT162b2 and CoronaVac) in adults with cancer: a territory-wide cohort study

Wei Kang[1†], Jessica J. P. Shami[1†], Vincent K. C. Yan[1], Xuxiao Ye[1], Joseph E. Blais[6], Xue Li[1,2,3], Victor H. F. Lee[4], Celine S. L. Chui[2,5,6], Francisco T. T. Lai[1,2], Eric Y. F. Wan[1,2,7], Carlos K. H. Wong[1,2,7], Ian C. K. Wong[1,2,8,9,10*] and Esther W. Chan[1,2,9,10*]

Abstract

Background: The World Health Organization has defined a list of adverse events of special interest (AESI) for safety surveillance of vaccines. AESI have not been adequately assessed following COVID-19 vaccination in patients with cancer contributing to vaccine hesitancy in this population. We aimed to evaluate the association between BNT162b2 and CoronaVac vaccines and the risk of AESI in adults with active cancer or a history of cancer.

Patients and methods: We conducted a territory-wide cohort study using electronic health records managed by the Hong Kong Hospital Authority and vaccination records provided by the Department of Health. Patients with a cancer diagnosis between January 1, 2018, and September 30, 2021, were included and stratified into two cohorts: active cancer and history of cancer. Within each cohort, patients who received two doses of BNT162b2 or CoronaVac were 1:1 matched to unvaccinated patients using the propensity score. Cox proportional hazards regression was used to estimate hazard ratios (HR) and 95% confidence intervals (CIs) for AESI 28 days after the second vaccine dose.

Results: A total of 74,878 patients with cancer were included (vaccinated: 25,789 [34%]; unvaccinated: 49,089 [66%]). Among patients with active cancer, the incidence of AESI was 0.31 and 1.02 per 10,000 person-days with BNT162b2 versus unvaccinated patients and 0.13 and 0.88 per 10,000 person-days with CoronaVac versus unvaccinated patients. Among patients with history of cancer, the incidence was 0.55 and 0.89 per 10,000 person-days with BNT162b2 versus unvaccinated patients and 0.42 and 0.93 per 10,000 person-days with CoronaVac versus unvaccinated patients. Neither vaccine was associated with a higher risk of AESI for patients with active cancer (BNT162b2: HR 0.30, 95% CI 0.08–1.09; CoronaVac: 0.14, 95% CI 0.02–1.18) or patients with history of cancer (BNT162b2: 0.62, 95% CI 0.30–1.28; CoronaVac: 0.45, 95% CI 0.21–1.00).

Conclusions: In this territory-wide cohort study of patients with cancer, the incidence of AESI following vaccination with two doses of either BNT162b2 or CoronaVac vaccines was low. The findings of this study can reassure clinicians

†Wei Kang and Jessica J. P. Shami are joint first authors

*Correspondence: wongick@hku.hk; ewchan@hku.hk

[1] Centre for Safe Medication Practice and Research, Department of Pharmacology and Pharmacy, General Office, L02-56 2/F, Laboratory Block, LKS Faculty of Medicine, The University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong SAR, China
Full list of author information is available at the end of the article

and patients with cancer about the overall safety of BNT162b2 and CoronaVac in patients with cancer, which could increase the COVID-19 vaccination rate in this vulnerable group of patients.

Keywords: COVID-19, Vaccine, Safety, Adverse events of special interest (AESI), BNT162b2, CoronaVac, Cancer

Introduction

Public health agencies recommend that patients with cancer should be prioritized for COVID-19 vaccination [1–3]. Currently, the safety of COVID-19 vaccines remains a concern, especially among the elderly and immunocompromised patients such as patients with cancer [4]. This has led to lower rates of vaccine uptake in patients with cancer in some regions including Hong Kong [5–7]. However, the available observational studies of BNT162b2 (mRNA, Pfizer-BioNTech) and CoronaVac (inactivated, Sinovac) vaccines in patients with cancer have only assessed common adverse events, for example headache and fever; have small sample sizes and are therefore unable to detect uncommon or rare adverse events of special interest (AESI); and do not have suitable between-individual comparisons, since they either have no comparator group or use a comparator group of healthy adults without cancer [8–15]. Furthermore, most patients with cancer were excluded from pivotal clinical trials of BNT162b2 and CoronaVac as their cancer treatments may suppress or impair the immune system [16–18]. Our study aimed to describe and assess the risk of AESI, as defined by the World Health Organization, among patients with active cancer and a history of cancer who received vaccination with BNT162b2 or CoronaVac.

Methods

Data sources

This study used electronic health records provided by the Hospital Authority and linked vaccination records provided by the Department of Health in Hong Kong. The linked records have been previously used to evaluate the safety of COVID-19 vaccines [19–23]. Diagnosis records were identified using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) diagnosis codes (Additional file 1: Table S1), and prescription records were identified using British National Formulary (BNF) codes (Additional file 1: Table S2).

Study population

Patients with a cancer diagnosis record between January 1, 2018, and September 30, 2021, were identified. Since patients with cancer have a weaker immune response after COVID-19 vaccination, AESI outcomes were only evaluated following the second dose of the vaccine [24]. The index date was defined as the date of the second vaccine dose for patients who were vaccinated with either BNT162b2 or CoronaVac. For unvaccinated patients, the pseudo index date was selected from a corresponding vaccine recipient matched on age and sex. Patients younger than 18 years, hospitalized within 30 days before vaccination, or diagnosed with cancer on or after the first dose of vaccination were excluded. Patients who received only the first dose of the vaccine were also excluded. The study population was stratified into two mutually exclusive cohorts: patients with active cancer and patients with a history of cancer (Fig. 1). Active cancer patients were defined as those who had undergone any active cancer treatment or had a diagnosis of metastasis in the last 6 months before their first vaccine dose [25]. The remaining patients were considered as the history of cancer cohort.

Outcomes

The primary outcome of interest in this study was the incidence of 28-day AESI, defined by the World Health Organization as a list of important vaccine safety surveillance events. The list includes conditions such as acute respiratory distress syndrome, acute kidney injury, myocarditis, and thrombocytopenia (Additional file 1: Table S1) [26]. The secondary outcome was 28-day all-cause mortality. Patients were followed from the index date until a diagnosis of the outcome, death, 28 days after the index date, or the end of study period (September 30, 2021), whichever occurred first.

Statistical analysis

Baseline patient characteristics were presented as means (standard deviation) for continuous variables and frequencies (percentages) for categorical variables. To reduce confounding arising from differences in baseline characteristics between vaccinated and unvaccinated patients, propensity score (PS) matching was performed for each type of vaccine (both in active cancer and in history of cancer cohorts). Confounders included in the PS estimation included age, sex, smoking, obesity, index date, history of COVID-19 (history of positive PCR test), latest levels of white blood cells and neutrophils before vaccination, hospitalization, accident and emergency attendance, cancer type and site, comorbidities, and concomitant medication use (Additional file 1: Tables S3, S4). Patients who received BNT162b2 vaccine and unvaccinated patients were matched on a 1:1

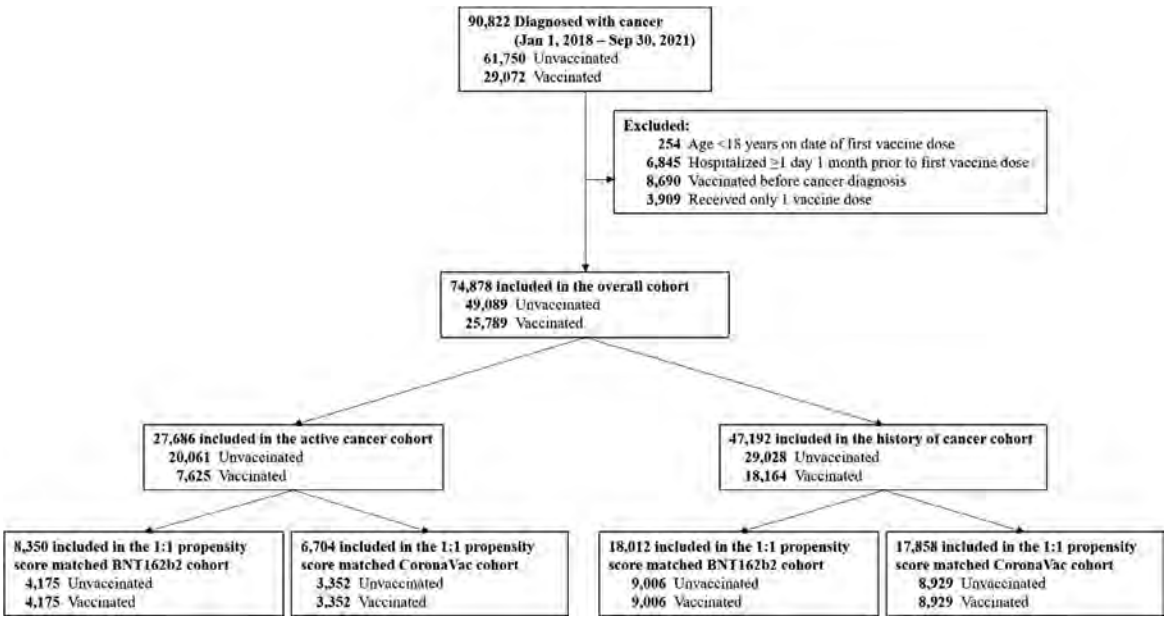


Fig. 1 Patient selection flowchart

ratio using nearest neighbor algorithm with a caliper of 0.01. The same matching procedure was performed for patients who received the CoronaVac vaccine. A standardized mean difference (SMD) of < 0.1 was considered acceptable.

The association of AESI with either BNT162b2 or CoronaVac vaccine among patients with cancer was estimated using Cox proportional hazards regression. The results were reported as hazard ratios (HR) with 95% confidence intervals (CIs). Subgroup analyses were performed on different age-groups, sex, and cancer types. Individuals who experienced severe adverse effects after the first dose would less likely accept the second dose, which could potentially introduce bias in the current two-dose analysis. Hence, a post hoc analysis was conducted to compare the cumulative incidence rate of AESI between patients who received one dose only and unvaccinated patients; chi-square test with a significance level of 0.05 was reported.

R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical analyses. The analyses were conducted by WK and cross-checked independently by JJPS and XY for quality assurance.

Results

We identified 90,822 patients with a cancer diagnosis between January 1, 2018, and September 30, 2021. After applying the exclusion criteria, 74,878 patients (25,789 active cancer cohort and 49,089 history of cancer cohort) were included (Fig. 1). After 1:1 PS matching, 15,054 patients with active cancer (4175 BNT162b2; 3352 CoronaVac; 7527 unvaccinated) and 35,870 patients with a history of cancer (9006 BNT162b2; 8929 CoronaVac; 17,935 unvaccinated) were included (Additional file 1: Tables S3, S4). All SMDs of the variables were < 0.1.

In the active cancer cohort, the incidence of AESI was 0.31 and 1.02 per 10,000 person-days for patients receiving BNT162b2 and matched unvaccinated patients, respectively; 0.13 and 0.88 per 10,000 person-days for patients receiving CoronaVac vaccine and matched unvaccinated patients, respectively (Table 1, Additional file 1: Table S5). In patients with a history of cancer, the incidence of AESI was 0.55 and 0.89 per 10,000 person-days for those who received BNT162b2 and matched unvaccinated patients, respectively; 0.42 and 0.93 per 10,000 person-days for patients who received CoronaVac and matched unvaccinated patients, respectively.

Patients who received BNT162b2 or CoronaVac were not at a higher risk of AESI compared to unvaccinated patients in the active cancer cohort [BNT162b2 HR: 0.30 (95% CI 0.08–1.09); CoronaVac HR: 0.14 (95% CI 0.02–1.18)]. Similarly, patients who received BNT162b2 or CoronaVac were not at a higher risk of AESI compared to unvaccinated patients in the history of cancer cohort [BNT162b2 HR: 0.62 (95% CI 0.30–1.28), CoronaVac HR: 0.45 (95% CI 0.21–1.00)] (Table 1). Results were

Table 1 Risk of 28-day post-vaccination AESI in vaccinated and unvaccinated patients with cancer after propensity score matching

Cohorts	BNT162b2				CoronaVac			
	Events/follow-up time (person-days)/incidence (per 10,000 person-days)		Hazard ratio[a] (95% CI)	*P* value	Events/follow-up time (person-days)/incidence (per 10,000 person-days)		Hazard ratio (95% CI)	*P* value
	Unvaccinated (*N* = 4175)	Vaccinated (*N* = 4175)			Unvaccinated (*N* = 3352)	Vaccinated (*N* = 3352)		
Active cancer								
All	10/97586/1.02	3/97588/0.31	0.30 (0.08–1.09)	0.07	7/79150/0.88	1/78204/0.13	0.14 (0.02–1.18)	0.07
Male	7/24853/2.82	1/23796/0.42	0.15 (0.02–1.22)	0.08	3/23137/1.30	1/22894/0.44	–	–
Female	3/72733/0.41	2/73792/0.27	0.65 (0.11–3.92)	0.64	4/56013/0.71	0/55310/0	–	–
Age < 60 years	1/50970/0.20	2/51891/0.39	–	–	1/33853/0.30	1/33410/0.30	–	–
Age ≥ 60 year	9/46616/1.93	1/45697/0.22	0.11 (0.01–0.90)	< 0.05	6/45297/1.32	0/44794/0	–	–
Solid tumor	8/90350/0.89	3/90634/0.33	0.37 (0.10–1.41)	0.15	7/75622/0.93	1/74229/0.13	0.15 (0.02–1.19)	0.07
Hematological malignancy	2/7236/2.76	0/6954/0	–	–	0/3528/0	0/3975/0	–	–
	Unvaccinated (*N* = 9006)	Vaccinated (*N* = 9006)			Unvaccinated (*N* = 8929)	Vaccinated (*N* = 8929)		
History of cancer								
All	19/213182/0.89	12/216640/0.55	0.62 (0.30–1.28)	0.20	20/214652/0.93	9/213033/0.42	0.45 (0.21–1.00)	< 0.05
Male	13/94638/1.37	4/96765/0.41	0.30 (0.10–0.92)	< 0.05	14/107447/1.30	8/106183/0.75	0.58 (0.24–1.38)	0.22
Female	6/118544/0.51	8/119875/0.67	1.32 (0.46–3.81)	0.61	6/107205/0.56	1/106850/0.09	0.17 (0.02–1.39)	0.10
Age < 60 years	7/99158/0.71	4/99283/0.40	0.57 (0.17–1.96)	0.37	5/77057/0.65	0/74134/0	–	–
Age ≥ 60 years	12/114024/1.05	8/117357/0.68	0.65 (0.27–1.60)	0.35	15/137595/1.09	9/138899/0.65	0.59 (0.26–1.36)	0.22
Solid tumor	18/197988/0.91	10/202083/0.49	0.55 (0.25–1.18)	0.12	19/203168/0.94	8/201881/0.40	0.42 (0.19–0.97)	< 0.05
Hematological malignancy	1/15194/0.66	2/14557/1.37	–	–	1/11484/0.87	1/11152/0.90	–	–

AESI: adverse events of special interest

[a] Hazard ratios are not shown if total events are less than 5 in each subgroup

consistent in all subgroup analyses; vaccinated patients had no increased risk of AESI compared to unvaccinated patients.

Among patients with active cancer, there were two deaths in the BNT162b2 group versus 22 among matched unvaccinated patients; and no deaths in the CoronaVac group versus 12 among matched unvaccinated patients. Among patients with a history of cancer, there was one death in the BNT162b2 group versus 13 among matched unvaccinated patients, and 2 deaths in the CoronaVac group versus 17 among matched unvaccinated patients (Additional file 1: Table S5). In the post hoc analysis, the cumulative incidence rate of AESI was not significantly different between patients who received one dose only, compared to unvaccinated patients (0.5% one-dose only and 0.4% unvaccinated, $\chi^2 = 0.63$, $p = 0.43$; Additional file 1: Table S6).

Discussion

The low rate of COVID-19 vaccine uptake in our study appears to reflect safety concerns among patients with cancer in Hong Kong. On September 30, 2021, our data showed that the overall vaccination rate in Hong Kong was 58.8%, while among patients with cancer it was only 30.2%. Our study provides reassurance that patients with cancer are not at an increased risk of AESI or death following two doses of either BNT162b2 or CoronaVac.

Several small observational studies have evaluated the safety of BNT162b2 or CoronaVac vaccines in patients with cancer [8–15]. All of those studies evaluated short-term common adverse events, including pain and swelling at the injection site, headache, fever, and diarrhea. However, no previous study examined AESI as an outcome and none included both patients with active cancer and patients with a history of cancer. To date, the largest study included 816 patients with active cancer and 274 healthcare workers from a single institution in Italy [9]. However, the comparator group comprised healthy individuals with no cancer diagnosis.

To our knowledge, this is the first study to report on all AESI and to evaluate the association between BNT162b2 and CoronaVac and the risk of AESI among patients with active cancer or history of cancer. Our study is also the first and largest territory-wide cohort

study that reports on 25,789 patients vaccinated with either BNT162b2 or CoronaVac. Furthermore, our study provides reassuring safety data on these two vaccines in a predominantly Asian population.

This study has several limitations. Firstly, patients in relatively better health or with better prognosis are more likely to get vaccinated, which may lead to a healthy user bias. Therefore, PS matching was used to minimize baseline confounding. Secondly, most AESI that were examined tend to be severe and relatively rare (< 1/1000 person-years) [27]. As a result, we would have been unable to detect a small increase in AESI risk. Nevertheless, the findings are still reassuring since the number of AESI events was small. Finally, since patients who only received the first dose of the vaccine were excluded, this could bias the current two-dose analysis. Nevertheless, our post hoc analysis did not show any significant difference in the cumulative incidence rate of AESI between patients receiving one dose only compared to unvaccinated patients; hence, this is unlikely to bias our findings [28, 29]. Future studies with a longer follow-up period are needed to further inform about potential longer-term risks.

Conclusion

In Hong Kong, the vaccination rate among patients with cancer is relatively low. In the present study, there was no increased risk of AESI following two doses of either BNT162b2 or CoronaVac vaccines among patients with active cancer or a history of cancer. The findings of this study can reassure clinicians and patients about the overall short-term safety of BNT162b2 and CoronaVac in patients with cancer, which could increase the COVID-19 vaccination rate in this vulnerable group of patients.

Abbreviations

AESI: Adverse events of special interest; ICD-9-CM: International Classification of Diseases, Ninth Revision, Clinical Modification; BNF: British National Formulary; PS: Propensity score; HR: Hazard ratio; CI: Confidence interval; SMD: Standardized mean difference.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13045-022-01265-9.

Additional file 1. Supplementary figures.

Acknowledgements

We thank colleagues from the Drug Office of the Department of Health and from the Hospital Authority for providing vaccination and clinical data. We also thank Bernard Chan for administrative and technical assistance.

Author contributions

WK, JJPS, ICKW, and EWC contributed to conception and design of the study and acquisition, analysis, or interpretation of data; WK and JJPS drafted the manuscript and performed statistical analysis; all authors helped in critical revision of the manuscript for important intellectual content; ICKW and EWC provided administrative, technical, or material support and supervised the study. All authors read and approved the final manuscript.

Funding

This work was supported by a research grant from the Food and Health Bureau, the Government of the Hong Kong Special Administrative Region (Grant No. COVID19F01). FTTL and ICKW's post were partly funded by $D^2$4H; hence, this work was partly supported by AIR@InnoHK administered by Innovation and Technology Commission. The study sponsors were not directly involved in the design of the study, the collection, analysis, and interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication.

Availability of data and materials

The datasets analyzed during the current study are not publicly available.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (Reference Number: UW21-149) and the Department of Health Ethics Committee (LM21/2021). Informed patient consent was not required as the data used in this study were anonymized.

Consent for publication

Not applicable.

Competing interests

XL has received internal funding from the University of Hong Kong; consultancy fees from Merck Sharp & Dohme; research and educational grants from Janssen and Pfizer; research grants from the Food and Health Bureau of the Government of the Hong Kong Special Administrative Region; all of which are outside this work. CSLC has received personal fees from Prime Vigilance; Hong Kong Innovation and Technology Commission from Pfizer, IQVIA, and Amgen; research grants from the Food and Health Bureau of the Hong Kong Government of the Hong Kong Special Administrative Region; all of which are outside this work. FTTL has received the RGC Postdoctoral Fellowship from the Hong Kong Research Grants Council; research grants from the Food and Health Bureau of the Government of the Hong Kong Special Administrative Region; both of which are outside this work. EYFW has received research grants from the Hong Kong Research Grants Council; research grants from the Food and Health Bureau of the Government of the Hong Kong Special Administrative Region; both of which are outside this work. CKHW reports receipt of research funding from the EuroQoL Group Research Foundation, the Hong Kong Research Grants Council, and the Hong Kong Health and Medical Research Fund; all of which are outside this work. ICKW has received research supports from Amgen, Bayer, Bristol-Myers Squibb, GSK, Janssen, Novartis, Pfizer, the Hong Kong Research Grants Council, the Food and Health Bureau of the Government of the Hong Kong Special Administrative Region; the National Health and Medical Research Council in Australia; National Institute for Health Research in England, European Commission, speaker fees from Janssen and Medice in the previous three years; and is an independent non-executive director of Jacobson Medical in Hong Kong; all of which are outside this work. EWC has received research funds and grants from Research Grants Council (RGC, Hong Kong), Research Fund Secretariat of the Food and Health Bureau, Narcotics Division of the Security Bureau of the Hong Kong Special Administrative Region, National Natural Science Fund of China, Amgen, Bayer, Bristol-Myers Squibb, Janssen, Pfizer, Takeda, Wellcome Trust; and reports honorarium from Hospital Authority; all of which are outside this work. All other authors declare no competing interests.

Author details
[1]Centre for Safe Medication Practice and Research, Department of Pharmacology and Pharmacy, General Office, L02-56 2/F, Laboratory Block, LKS Faculty of Medicine, The University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong SAR, China. [2]Laboratory of Data Discovery for Health, Hong Kong SAR, China. [3]Department of Medicine, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China. [4]Department of Clinical Oncology, LKS Faculty of Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong SAR, China. [5]School of Nursing, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China. [6]School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China. [7]Department of Family Medicine and Primary Care, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China. [8]Research Department of Practice and Policy, School of Pharmacy, University College London, London, UK. [9]Department of Pharmacy, The University of Hong Kong-Shenzhen Hospital, Shenzhen, China. [10]The University of Hong Kong Shenzhen Institute of Research and Innovation, Shenzhen, China.

Received: 31 January 2022 Accepted: 14 April 2022
Published online: 19 May 2022

References

1. Al-Quteimat OM, Amer AM. The impact of the COVID-19 pandemic on cancer patients. Am J Clin Oncol. 2020;43(6):452–5.
2. Centre for Health Protection, Hong Kong Department of Health. Interim Guidance Notes On Common Medical Diseases and COVID-19 Vaccination In Primary Care Settings [Internet]. Hong Kong 2021 [updated 2021 November 24; cited 2022 January 25]. https://www.covidvaccine.gov.hk/pdf/Guidance_Notes.pdf.
3. Centers for Disease Control and Prevention. COVID-19 Vaccines for Moderately or Severely Immunocompromised People [Internet]. 2022 [updated 2022 January 7; cited 2022 January 25]. https://www.cdc.gov/coronavirus/2019-ncov/vaccines/recommendations/immuno.html.
4. Xiao J, Cheung JK, Wu P, Ni MY, Cowling BJ, Liao Q. Temporal changes in factors associated with COVID-19 vaccine hesitancy and uptake among adults in Hong Kong: serial cross-sectional surveys. Lancet Reg Health West Pac. 2022;23:100441.
5. Chun JY, Kim SI, Park EY, Park SY, Koh SJ, Cha Y, et al. Cancer patients' willingness to take COVID-19 vaccination: a nationwide multicenter survey in Korea. Cancers (Basel). 2021;13(15):3883.
6. Chan WL, Ho YT, Wong CK, Choi HC, Lam KO, Yuen KK, et al. Acceptance of COVID-19 vaccination in cancer patients in Hong Kong: approaches to improve the vaccination rate. Vaccines (Basel). 2021;9(7):792.
7. Khiari H, Cherif I, M'Ghirbi F, Mezlini A, Hsairi M. COVID-19 vaccination acceptance and its associated factors among cancer patients in Tunisia. Asian Pac J Cancer Prev. 2021;22(11):3499–506.
8. So ACP, McGrath H, Ting J, Srikandarajah K, Germanou S, Moss C, et al. COVID-19 vaccine safety in cancer patients: a single centre experience. Cancers (Basel). 2021;13(14):3573.
9. Di Noia V, Pimpinelli F, Renna D, Barberi V, Maccallini MT, Gariazzo L, et al. Immunogenicity and safety of COVID-19 vaccine BNT162b2 for patients with solid cancer: a large cohort prospective study from a single institution. Clin Cancer Res. 2021;27(24):6815–23.
10. Ligumsky H, Safadi E, Etan T, Vaknin N, Waller M, Croll A, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine among actively treated cancer patients. J Natl Cancer Inst. 2021;114:203–9.
11. Karacin C, Eren T, Zeynelgil E, Imamoglu GI, Altinbas M, Karadag I, et al. Immunogenicity and safety of the coronavac vaccine in patients with cancer receiving active systemic therapy. Future Oncol. 2021;17(33):4447–56.
12. Monin L, Laing AG, Munoz-Ruiz M, McKenzie DR, Barrio DMD, I, Alaguthurai T, et al. Safety and immunogenicity of one versus two doses of the COVID-19 vaccine BNT162b2 for patients with cancer: interim analysis of a prospective observational study. Lancet Oncol. 2021;22(6):765–78.
13. Trillo Aliaga P, Trapani D, Sandoval JL, Crimini E, Antonarelli G, Vivanet G, et al. Safety of COVID-19 mRNA vaccines in patients with cancer enrolled in early-phase clinical trials. Cancers (Basel). 2021;13(22):5829.
14. Kian W, Zemel M, Kestenbaum EH, Rouvinov K, Alguayn W, Levitas D, et al. Safety of the BNT162b2 mRNA COVID-19 vaccine in oncologic patients undergoing numerous cancer treatment options: a retrospective single-center study. Medicine (Baltimore). 2022;101(2):e28561.
15. Tamura T, Ninomiya K, Kubo T, Kuyama S, Tachibana S, Inoue K, et al. Short-term safety of an anti-severe acute respiratory syndrome coronavirus 2 messenger RNA vaccine for patients with advanced lung cancer treated with anticancer drugs: a multicenter, prospective, observational study. Thorac Cancer. 2022;13(3):453–9.
16. Corti C, Curigliano G. Commentary: SARS-CoV-2 vaccines and cancer patients. Ann Oncol Off J Eur Soc Med Oncol. 2021;32(4):569–71.
17. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020;383(27):2603–15.
18. Palacios R, Batista AP, Albuquerque CSN, Patiño EG, Santos JdP, Conde MTRP, et al. Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in Brazil: The PROFISCOV Study. SSRN; 2021.
19. Wan EYF, Chui CSL, Lai FTT, Chan EWY, Li X, Yan VKC, et al. Bell's Palsy following vaccination with mRNA (BNT162b2) and inactivated (CoronaVac) SARS-CoV-2 vaccines: a case series and nested case-control study. Lancet Infect Dis. 2022;22(1):64–72.
20. Lai FTT, Huang L, Chui CSL, Wan EYF, Li X, Wong CKH, et al. Multimorbidity and adverse events of special interest associated with Covid-19 vaccines in Hong Kong. Nat Commun. 2022;13(1):411.
21. Lai FTT, Li X, Peng K, Huang L, Ip P, Tong X, et al. Carditis after COVID-19 vaccination with a messenger RNA vaccine and an inactivated virus vaccine : a case-control study. Ann Intern Med. 2022;175(3):362–70.
22. Wan EYF, Chui CSL, Wang Y, Ng VWS, Yan VKC, Lai FTT, et al. Herpes zoster related hospitalization after inactivated (CoronaVac) and mRNA (BNT162b2) SARS-CoV-2 vaccination: a self-controlled case series and nested case-control study. Lancet Reg Health West Pac. 2022;21:100393.
23. Lai FTT, Huang L, Peng K, Li X, Chui CSL, Wan EYF, et al. Post-Covid-19-vaccination adverse events and healthcare utilization among individuals with or without previous SARS-CoV-2 infection. J Intern Med. 2022.
24. Massarweh A, Eliakim-Raz N, Stemmer A, Levy-Barda A, Yust-Katz S, Zer A, et al. Evaluation of seropositivity following BNT162b2 messenger RNA vaccination for SARS-CoV-2 in patients undergoing treatment for cancer. JAMA Oncol. 2021;7(8):1133–40.
25. Khorana AA, Noble S, Lee AYY, Soff G, Meyer G, O'Connell C, et al. Role of direct oral anticoagulants in the treatment of cancer-associated venous thromboembolism: guidance from the SSC of the ISTH. J Thromb Haemost. 2018;16(9):1891–4.
26. World Health Organization. COVID-19 Vaccines: Safety Surveillance Manual [Internet]. [updated 2020; cited 2022 January 25]. https://apps.who.int/iris/handle/10665/338400. License: CC BY-NC-SA 3.0 IGO.
27. Li XT, Ostropolets A, Makadia R, Shoaibi A, Rao G, Sena AG, et al. Characterising the background incidence rates of adverse events of special interest for covid-19 vaccines in eight countries: multinational network cohort study. BMJ. 2021;373:n1435.
28. Li X, Tong X, Yeung WWY, Kuan P, Yum SHH, Chui CSL, et al. Two-dose COVID-19 vaccination and possible arthritis flare among patients with rheumatoid arthritis in Hong Kong. Ann Rheum Dis. 2022;81(4):564–8.
29. Food and Health Bureau The Government of the Hong Kong Special Administrative Region. Report on Evaluation of Safety, Efficacy and Quality of Comirnaty COVID-19 mRNA Vaccine (BNT162b2) Concentrate for Dispersion for Injection [Internet]. [updated 2022 January 12; cited 2022 April 7]. https://www.fhb.gov.hk/download/our_work/health/201200/e_evaluation_report.pdf.

Publisher's Note

4.3. Terceira dose da CoronaVac promove resposta imune robusta em pacientes com doenças reumáticas

A terceira dose da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, induz resposta imune elevada contra a Covid-19 em pacientes com doenças reumáticas autoimunes, com produção de anticorpos em mais de 90% dos indivíduos. Esta é a principal conclusão de um trabalho realizado no Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, publicado na revista Annals of the Rheumatic Diseases do British Medical Journal.

Os cientistas incluíram no estudo 597 pacientes adultos com doenças reumáticas autoimunes, assim como um grupo controle com 199 pessoas saudáveis, sendo a idade média e a distribuição de gênero comparáveis em ambos os grupos. Todos os participantes haviam sido vacinados com duas doses de CoronaVac e receberam a terceira dose seis meses após a segunda. Pacientes com doença controlada suspenderam as medicações imunossupressoras durante o período de vacinação.

Entre os pacientes, a taxa de soroconversão de anticorpos IgG contra o SARS-CoV-2 aumentou significativamente com a terceira dose, de 60% para 93% um mês após a aplicação. Da mesma forma, a positividade dos anticorpos neutralizantes aumentou de 38% antes da terceira dose para 81,4% decorridos 30 dias da dose de reforço. O mesmo padrão foi observado para o grupo controle.

A terceira dose da CoronaVac também elevou a resposta imune de pacientes que estavam sem produzir anticorpos seis meses após a segunda dose. Com o reforço, a soroconversão chegou a 80,5% para anticorpos IgG e 59,1% para anticorpos neutralizantes.

É importante ressaltar que a queda na soroconversão seis meses após a segunda dose já foi comprovada pela ciência em todas as vacinas contra a Covid-19 atualmente em uso, e está relacionada à dinâmica do vírus SARS-CoV-2, não à eficácia dos imunizantes.

Análises adicionais revelaram que fatores como idade avançada, diagnóstico de vasculite e uso dos medicamentos prednisona (corticoide) e micofenolato de mofetila (para prevenir rejeição de transplantes) estavam associados à redução da positividade dos anticorpos IgG. Além disso, o uso dos fármacos prednisona, abatacepte, belimumab e rituximabe estavam relacionados à menor produção de anticorpos neutralizantes.

De acordo com os pesquisadores, o trabalho reforça a importância da terceira dose para pessoas com doenças reumáticas autoimunes. "Este estudo fornece novas evidências de um aumento substancial na resposta imune com uma dose adicional da CoronaVac administrada seis meses após duas doses da mesma vacina inativada, em uma grande coorte prospectiva controlada de pacientes".

Publicado em: 11/03/2022

CLINICAL SCIENCE

Increment of immunogenicity after third dose of a homologous inactivated SARS-CoV-2 vaccine in a large population of patients with autoimmune rheumatic diseases

Nádia Emi Aikawa ,[1] Leonard de Vinci Kanda Kupa,[1] Ana Cristina Medeiros-Ribeiro,[1] Carla Goncalves Schahin Saad,[1] Emily Figueiredo Neves Yuki,[1] Sandra Gofinet Pasoto,[1] Priscila Tagliaferro Rojo,[1] Rosa Maria Rodrigues Pereira,[1] Samuel Katsuyuki Shinjo,[1] Percival Degrava Sampaio-Barros,[1] Danieli Castro Oliveira Andrade ,[1] Ari Stiel Radu Halpern,[1] Ricardo Fuller,[1] Fernando Henrique Carlos Souza,[1] Lissiane Karine Noronha Guedes,[1] Ana Paula Luppino Assad,[1] Julio Cesar Bertacini de Moraes,[1] Michelle Remiao Ugolini Lopes,[1] Victor Adriano de Oliveira Martins,[1] Lorena Betancourt,[1] Carolina Torres Ribeiro,[1] Lucas Peixoto Sales,[1] Isabela Maria Bertoglio,[1] Virginia Lucia Nazario Bonoldi,[1] Renata Lys Pinheiro Mello,[1] Gustavo Guimaraes Moreira Balbi,[1] Ana Marli Christovam Sartori,[2] Leila Antonangelo,[3] Clóvis Artur Silva,[4] Eloisa Bonfa [1]

Handling editor Josef S Smolen

For numbered affiliations see end of article.

Correspondence to
Eloisa Bonfa, Rheumatology Division, Hospital das Clinicas, Faculdade de Medicina, Universidade de Sao Paulo - Av. Dr. Arnaldo, 455, sala 3190 - Cerqueira César, São Paulo – SP – Brazil, ZIP-code 01246-903
E-mail – eloisa.bonfa@hc.fm.usp.br, Sao Paulo, SP, Brazil; eloisa.bonfa@hc.fm.usp.br

CAS and EB contributed equally.

Received 30 December 2021
Accepted 28 February 2022

To cite: Aikawa NE, Kupa LdVK, Medeiros-Ribeiro AC, *et al*. *Ann Rheum Dis* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/annrheumdis-2021-222096

ABSTRACT

Objective To determine the immunogenicity of the third dose of CoronaVac vaccine in a large population of patients with autoimmune rheumatic diseases (ARD) and the factors associated with impaired response.

Methods Adult patients with ARD and age-balanced/sex-balanced controls (control group, CG) previously vaccinated with two doses of CoronaVac received the third dose at D210 (6 months after the second dose). The presence of anti-SARS-CoV-2 S1/S2 IgG and neutralising antibodies (NAb) was evaluated previously to vaccination (D210) and 30 days later (D240). Patients with controlled disease suspended mycophenolate mofetil (MMF) for 7 days or methotrexate (MTX) for 2 weekly doses after vaccination.

Results ARD (n=597) and CG (n=199) had comparable age (p=0.943). Anti-S1/S2 IgG seropositivity rates significantly increased from D210 (60%) to D240 (93%) (p<0.0001) in patients with ARD. NAb positivity also increased: 38% (D210) vs 81.4% (D240) (p<0.0001). The same pattern was observed for CG, with significantly higher frequencies for both parameters at D240 (p<0.05). Multivariate logistic regression analyses in the ARD group revealed that older age (OR=0.98, 95% CI 0.96 to 1.0, p=0.024), vasculitis diagnosis (OR=0.24, 95% CI 0.11 to 0.53, p<0.001), prednisone ≥5 mg/day (OR=0.46, 95% CI 0.27 to 0.77, p=0.003), MMF (OR=0.30, 95% CI 0.15 to 0.61, p<0.001) and biologics (OR=0.27, 95% CI 0.16 to 0.46, p<0.001) were associated with reduced anti-S1/S2 IgG positivity. Similar analyses demonstrated that prednisone ≥5 mg/day (OR=0.63, 95% CI 0.44 to 0.90, p=0.011), abatacept (OR=0.39, 95% CI 0.20 to 0.74, p=0.004), belimumab

Key messages

What is already known about this subject?

- The waning of immunity elicited by vaccines was reported to be associated with breakthrough cases in different countries, driven predominantly by the Delta variant and now the threat of Omicron variant of SARS-CoV-2.
- Patients with autoimmune rheumatic diseases (ARD) are at high risk of severe COVID-19 and are known to have reduced primary vaccination response.
- There is evidence on the efficacy of a third dose in increasing humoral response and protective effect in the general population.

What does this study add?

- The third dose of COVID-19 vaccine results in a robust immunogenicity response for patients with ARD overall and for those who were COVID-19 seronegative at 6 months post primary full vaccination.

How might this impact on clinical practice or future developments?

- The third dose of anti-SARS-CoV-2 vaccine should be strongly recommended for patients with ARD 6 months after primary vaccination as an excellent strategy to improve waning of vaccine-induced COVID-19 immunogenicity over time.

(OR=0.29, 95% CI 0.13 to 0.67, p=0.004) and rituximab (OR=0.11, 95% CI 0.04 to 0.30, p<0.001) were negatively associated with NAb positivity. Further evaluation of COVID-19 seronegative ARD at D210 demonstrated prominent increases in positivity rates at D240 for anti-S1/S2 IgG (80.5%) and NAb (59.1%) (p<0.0001).

Conclusions We provide novel data on a robust response to the third dose of CoronaVac in patients with ARD, even in those with prevaccination COVID-19 seronegative status. Drugs implicated in reducing immunogenicity after the regular two-dose regimen were associated with non-responsiveness after the third dose, except for MTX.

Trial registration number NCT04754698.

INTRODUCTION

The COVID-19 pandemic is still a global problem and mass vaccination has been crucial for the waning of the epidemic worldwide. Until now, almost one-third of the Brazilian population has been vaccinated with CoronaVac. There is real-world evidence on the high effectiveness of CoronaVac in reducing hospitalisations and deaths related to SARS-CoV-2 infection in 10.2 million people in Chile.[1]

However, even in countries with high vaccination rates, breakthrough cases in both vaccinated and unvaccinated persons are increasingly being reported,[2–4] driven predominantly by the SARS-CoV-2 Delta variant[5–7] and more recently by the Omicron variant. The Advisory Committee on Immunization Practices of the Centers for Disease Control and Prevention (CDC) recommended a third dose of COVID-19 vaccine to high-risk groups, including immunocompromised individuals, to address potential waning immunity against the SARS-CoV-2 variants, with acceptable safety profile.[8–10]

Immunosuppressed patients with autoimmune rheumatic diseases (ARD) are generally under increased risk of severe COVID-19.[11 12] We have previously demonstrated that two doses of CoronaVac elicited moderate humoral response in patients with naïve ARD, with a parallel major decrease in incident COVID-19 cases post immunisation.[10] A more robust response was observed in individuals with ARD pre-exposed to COVID-19, with a high plateau of response after a single CoronaVac dose.[13] Neutralising antibodies' (NAb) response dynamics in patients who have recovered from COVID-19 may vary greatly.[14] We further demonstrated a substantial decline of anti-SARS-CoV-2 antibodies in patients with ARD 6 months after a two-dose schedule of CoronaVac.[15]

There is increasing evidence on the efficacy of a third dose of vaccine in enhancing protective effect.[16–19] However, data on CoronaVac are limited. A study in healthy adults demonstrated a strong humoral booster following an additional dose administered 8 months after the primary schedule, indicating an efficient recalling of SARS-CoV-2-specific immune memory.[20] The efficacy of a third dose of messenger RNA (mRNA) vaccine was also recently reported in the general population in Israel, with more than 90% reduction in COVID-19-related hospitalisations and deaths.[21] In subjects ≥60 years, a third dose of BNT162b2 reduced severe infection rate by a factor of almost 20.[19] Furthermore, a third dose of the viral vector ChAdOx1 nCoV-19 vaccine could boost antibody and T cell responses in healthy volunteers.[22] Among immunocompromised populations, a significant proportion of patients (30%–50%) with inadequate response to two mRNA vaccine doses seroconverted after an additional dose,[23–30] and insufficient response was mainly associated with higher degree of immunosuppression.[23 24]

Regarding ARD population, there is one case series reporting that a third dose of mRNA vaccine induced seroconversion in almost 90% of 17 patients with rheumatoid arthritis with minimal response to primary vaccination. Most of them discontinued disease modifying anti-rheumatic drugs (DMARD) temporarily to receive the third dose[17] and more data are necessary.

We therefore performed a prospective analysis of the immunogenicity and safety of a third CoronaVac dose, administered 6 months after the standard two-dose homologous schedule, in a large ARD population compared with an age-balanced and sex-balanced control group (CG). Possible factors associated with lack of humoral immune response after the third dose were secondarily assessed.

METHODS

Study design and population

We conducted a phase 4 prospective longitudinal study (CoronavRheum) at a large academic hospital in Sao Paulo, Brazil. All participants signed the written informed consent. Patients with ARD were diagnosed according to the international classification criteria for each disease[31–40] and were regularly followed at the outpatient rheumatology clinics. Subsequently, we invited subjects without ARD or immunosuppressive therapy as the CG. All subjects were ≥18 years old. CG was sex-balanced and age-balanced with patients with ARD (±5 years) at entry (1 control to 3 patients). All participants had previously received two doses of the inactivated vaccine CoronaVac (batch #20200412; Sinovac Life Sciences, Beijing, China) 28 days apart (first dose (D0): 9–17 February 2021; second dose (D28): 9–17 March 2021) and were recruited to receive the third dose from 13 September to 18 September 2021 (D210: 6 months later). The electronic charts of all patients under mycophenolate mofetil (MMF) or methotrexate (MTX) were reviewed for disease activity, 10 days before vaccination. Those with low disease activity/inactive disease at last visit (up to 2 months) were interviewed at D210 by a physician to confirm clinical status and to guide medication withdrawal after vaccination (1 week for MMF and 2 weekly doses for MTX). The exclusion criteria were third dose vaccination with any other SARS-CoV-2 vaccine, prior anaphylactic events to vaccines, immunisation with live virus in the last 4 weeks or inactivated vaccine in the last 2 weeks, Guillain-Barré syndrome, decompensated heart failure, demyelinating disease, COVID-19-related symptoms, acute febrile illness, or hospitalisation at vaccination day (figure 1). All participants had their first blood sample collected at D210 and the second sample collected at D240 (1 month after the third dose; from 14 October to 4 November 2021). Patients or the public were not involved in the design, or conduct, or reporting or dissemination plans of the study.

Primary and secondary outcomes

The primary outcome was humoral immunogenicity assessed by the presence of anti-SARS-CoV-2 S1/S2 IgG at D240 in all participants. The secondary outcomes were factor increase (FI) in anti-S1/S2 geometric mean titre (GMT) from D210 to D240, presence of NAb, NAb activity, and the influence of demographic data, ARD diagnosis and current therapy on anti-SARS-CoV-2 seropositivity at D240.

Serological assays

Serological assay consisted of total anti-SARS-CoV-2 S1/S2 IgG (chemiluminescent immunoassay by indirect ELISA; ETI-MAX 3000 equipment, LIAISON SARS-CoV-2 S1/S2 IgG

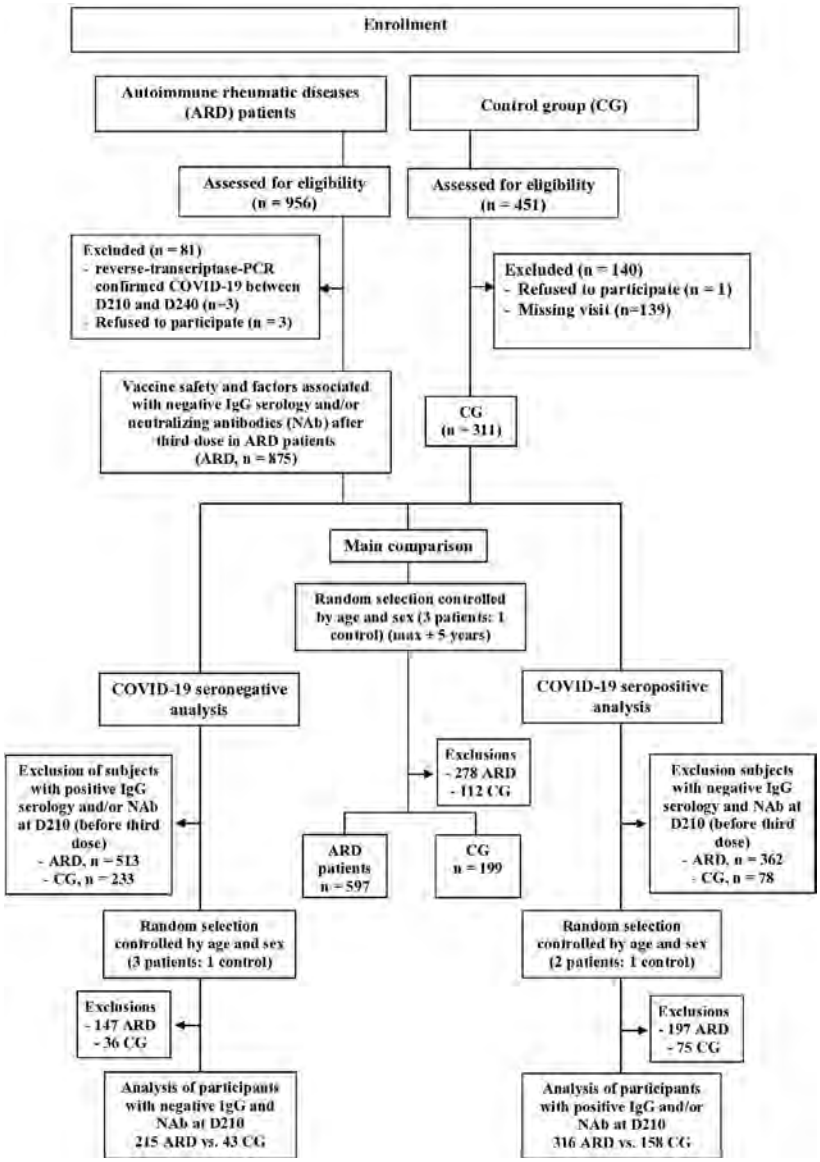


Figure 1 Flow chart diagram. The diagram shows the flow of eligible individuals, exclusions and analysed participants. Reasons for exclusions are given in the figure. D210, 6 months after second dose.

Kit, DiaSorin, Italy) and circulating NAb against SARS-CoV-2 using the SARS-CoV-2 sVNT Kit (GenScript, Piscataway, New Jersey, USA), following the manufacturer's instructions. Samples with 15.0 AU/mL or more for total IgG and with 30% or more inhibition in the neutralising assay were considered seropositive according to the manufacturer.[41] Quantitative results were reported, attributing the value of 1.9 AU/mL (half of the lower limit of quantification 3.8 AU/mL) to undetectable levels (<3.8 AU/mL) of IgG. NAb activity was calculated as median (IQR) only considering positive samples at D210 and D240.

Data and statistical analysis

Data were presented as number (percentage) for categorical variables and as mean±SD or median (IQR) for continuous variables. Comparisons were performed by χ^2 or Fisher's exact tests, as appropriate, for categorical variables, and by Student's t-test or Mann-Whitney test for continuous variables. Seropositivity rates of anti-S1/S2 IgG and NAb were presented as number (percentage) and were compared between groups (ARD and CG) and between timepoints (D210 vs D240) using repeated measures analysis of variance with two factors followed by Bonferroni's multiple comparisons in Napierian logarithm-transformed data for IgG. IgG titres were expressed as geometric mean with 95% CI. Multivariate logistic regression analyses were performed using as dependent variables seroconversion (SC)/NAb positivity at D240 and as independent variables those with $p<0.2$ in each univariate analysis. Statistical significance was defined as $p<0.05$. Most of the statistical analyses were performed using Statistical Package for the Social Sciences V.20.0.

RESULTS

A total of 956 patients with ARD and 451 controls were recruited in this protocol. After applying the exclusion criteria and random sampling (3 ARD to 1 CG), the final study groups consisted of

Table 1 Demographic data, clinical characteristics and treatment of patients with ARD and CG at D210, before the third dose of Sinovac-CoronaVac vaccine

	ARD (n=597)	CG (n=199)	P value
Demographic data			
Current age, years	50 (39–58)	49 (39–58)	0.9434
Age ≥60 years	117 (19.6)	40 (20.1)	0.918
Age at diagnosis, years	32 (23–43)	–	–
Disease duration, years	14 (8–21)	–	–
Female sex	411 (68.8)	137 (68.8)	>0.999
Caucasian race	293 (49.1)	84 (42.2)	0.213
ARD			
Rheumatoid arthritis	152 (25.5)	–	–
Axial spondyloarthritis	89 (14.9)	–	–
Psoriatic arthritis	66 (11.1)	–	–
Systemic lupus erythematosus	153 (25.6)	–	–
Systemic vasculitis	35 (5.8)	–	–
Idiopathic inflammatory myopathy	32 (5.4)	–	–
Systemic sclerosis	21 (3.5)	–	–
Primary Sjögren's syndrome	25 (4.2)	–	–
Primary antiphospholipid syndrome	23 (3.9)	–	–
Current therapies		–	–
Hydroxychloroquine	177 (29.6)	–	–
Sulfasalazine	52 (8.7)	–	–
Prednisone	199 (33.3)	–	–
Prednisone dose, mg/day	5 (5–10)	–	–
Immunosuppressive drugs	370 (62.0)	–	–
Methotrexate	172 (28.8)	–	–
Leflunomide	71 (11.9)	–	–
Mycophenolate mofetil	73 (12.2)	–	–
Azathioprine	62 (10.4)	–	–
Others*	33 (5.6)	–	–
Biologic agent	197 (33.0)	–	–
TNFi	87 (14.7)	–	–
Secukinumab	28 (4.7)	–	–
Tocilizumab	24 (4.0)	–	–
Abatacept	22 (3.7)	–	–
Belimumab	21 (3.5)	–	–
Rituximab	12 (2.0)	–	–
Ustekinumab	3 (1.0)	–	–

Results are expressed as median (IQR) and n (%).
Statistics: Fisher's exact test for categorical variables and Mann-Whitney test for continuous variables.
*Cyclophosphamide, ciclosporin, tacrolimus or tofacitinib.
ARD, autoimmune rheumatic diseases; CG, control group; D210, 6 months after second dose; TNFi, tumour necrosis factor inhibitor.

597 patients with ARD and 199 controls who collected blood sample and received the third dose of CoronaVac (D210) and returned after 30 days for blood collection (D240) (figure 1). Patients with ARD and CG were comparable with regard to median age and female sex (p>0.05) (table 1).

As part of standard of care, based on American College of Rheumatology guidance for COVID-19 vaccination,[42] 269 patients under MTX and 87 patients under MMF on low disease activity/inactivity were instructed to withhold these drugs for 2 weeks and 1 week, respectively.

Anti-S1/S2 IgG and NAb seropositivity rates in patients with ARD and in CG before (D210) and after (D240) the third dose of vaccine are presented in table 2. From D210 to D240, significant increase in anti-S1/S2 IgG and NAb positivity rates was observed for both ARD and CG (p<0.0001).

Anti-S1/S2 IgG GMT increased significantly from D210 to D240 in ARD (25.3 AU/mL vs 140.5 AU/mL, p<0.001) and in CG (47.9 AU/mL vs 253.8 AU/mL, p<0.001) (table 3). Expressive increments in NAb activity were also observed after the third dose of vaccine for both groups (p<0.0010).

The factors associated with IgG and NAb positivity after the third dose of vaccine (D240) in the ARD group are presented in table 4. The number of patients included in table 4 (n=875) comprised the total number of patients with ARD who were initially recruited and attended all study visits (received the third dose of CoronaVac at D210 and returned for blood collection at D240), before the random sampling (3 ARD to 1 CG) for immunogenicity analysis. The frequencies of patients with systemic vasculitis, prednisone, immunosuppressive drugs, MMF and biologic drug use, particularly abatacept, belimumab and rituximab, were significantly lower in IgG seropositive patients (p<0.05). NAb-positive patients at D240 presented lower frequencies of female sex, rheumatoid arthritis diagnosis, prednisone, immunosuppressive drugs and biologic drugs use, especially abatacept, belimumab and rituximab (p<0.05). On the other hand, spondyloarthritis, systemic sclerosis diagnosis and secukinumab use were associated with NAb positivity (p<0.05).

Multiple logistic regression analysis using IgG positivity at D240 as the dependent variable revealed that older age (OR=0.98, 95% CI 0.96 to 1.0, p=0.024), vasculitis (OR=0.24, 95% CI 0.11 to 0.53, p<0.001), prednisone ≥5 mg/day (OR=0.46, 95% CI 0.27 to 0.77, p=0.003), MMF use (OR=0.30, 95% CI 0.15 to 0.61, p<0.001) and biologic drug use (OR=0.27, 95% CI 0.16 to 0.46, p<0.001) were independently associated with anti-S1/S2 IgG response after the third dose (D240) in patients with ARD. For NAb analysis, multiple logistic regression revealed that prednisone ≥5 mg/day (OR=0.63, 95% CI 0.44 to 0.90, p=0.011), abatacept (OR=0.39, 95% CI 0.20 to 0.74, p=0.004), belimumab (OR=0.29, 95% CI 0.13 to 0.67,

Table 2 Anti-SARS-CoV-2 S1/S2 IgG and NAb seropositivity rates at baseline (D210) and 30 days after the third dose of Sinovac-CoronaVac vaccine (D240) in patients with ARD and in CG

	Anti-S1/S2 IgG positivity			NAb positivity		
Groups	D210	D240	P value	D210	D240	P value
ARD (n=597)	358 (60.0)	555 (93.0)	**<0.0001**	227 (38.0)	486 (81.4)	**<0.0001**
CG (n=199)	153 (76.9)	199 (100)	**<0.0001**	104 (52.3)	196 (98.5)	**<0.0001**
P value (ARD vs CG)	**<0.0001**	**<0.0001**		**0.0004**	**<0.0001**	

Frequencies of subjects with positive anti-SARS-CoV-2 S1/S2 IgG and NAb are expressed as number (%).
Positivity for anti-SARS-CoV-2 S1/S2 IgG was defined as postvaccination titre ≥15 AU/mL by Indirect ELISA (LIAISON SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy). Positivity for NAb was defined as a neutralising activity ≥30% (cPass sVNT Kit, GenScript, Piscataway, USA).
Frequencies of seropositivity were compared using McNemar's test for before and after comparisons and χ^2 or Fisher's exact tests for ARD vs CG comparisons.
p values <0.05 are highlighted in bold.
ARD, autoimmune rheumatic diseases; CG, control group; D210, 6 months after second dose; D240, 30 days after third dose; NAb, neutralising antibodies.

Table 3 GMT of anti-SARS-CoV-2 S1/S2 IgG and median percentage of neutralising activity at baseline (D210) and 30 days after the third dose of Sinovac-CoronaVac vaccine (D240) in patients with ARD and in CG

	Anti-S1/S2 IgG GMT (95% CI), AU/mL			Neutralising activity, median % (IQR)		
Groups	D210	D240	P value	D210	D240	P value
ARD (n=597)	25.3 (22.2–28.8)	140.5 (127.1–155.3)	**<0.001**	69.7 (45.9–92.5)	90.1 (70.2–96.6)	**<0.001**
CG (n=199)	47.9 (40.0–57.2)	253.8 (236.5–272.4)	**<0.001**	78.5 (61.1–94.6)	93.4 (83.6–97.0)	**<0.001**
P value (ARD vs CG)	**<0.001**	**<0.001**		**0.036**	**0.011**	

Percentage of neutralising activity of NAb is expressed as median (IQR) and anti-S1/S2 IgG antibody titres are expressed as geometric mean with 95% CI.
The mean behaviours of the Napierian ln-transformed IgG titres and NAb activity were compared between groups (ARD and CG) and timepoints (D210 and D240) using repeated measures analysis of variance with two factors followed by Bonferroni's multiple comparisons in ln-transformed data.
p values <0.05 are highlighted in bold.
ARD, autoimmune rheumatic diseases; CG, control group; D210, 6 months after second dose; D240, 30 days after third dose; GMT, geometric mean titres; ln, logarithm; NAb, neutralising antibodies.

p=0.004) and rituximab use (OR=0.11, 95% CI 0.04 to 0.30, p<0.001) were independently associated with NAb negativity after the third vaccine dose.

Further analysis of immune response to the third vaccine dose was performed considering COVID-19 seronegative (negative IgG and NAb at D210; n=215) and seropositive (positive IgG and/or NAb at D210; n=316) patients with ARD and the age-balanced and sex-balanced controls for each ARD group (figure 1). COVID-19 seronegative ARD and controls at D210 demonstrated prominent increase in IgG and NAb positivity rates as well as in GMT at D240 (p<0.0001). At D210, 120 patients with ARD were positive only for anti-S1/S2 IgG, 8 patients only for NAb and 187 patients for both. In COVID-19 seropositive ARD, significant GMT increase was observed from D210 to D240 (p<0.001), with 30.4% of the patients reaching the ceiling of the assay (>400 AU/mL). NAb activity also increased significantly at D240 (p<0.001) (table 5). Comparison of the FI-GMT from D210 to D240 between COVID-19 seronegative and seropositive ARD demonstrated a higher increment in the former group (11.3 (95% CI 9.5 to 13.4) vs 3.9 (95% CI 3.4 to 4.4), p<0.001).

Regarding safety, there were no differences between ARD and CG for any of the reported adverse events. The most frequently reported adverse events were local pain (23.8% vs 19.4%, p=0.238) and headache (13.1% vs 11.7%, p=0.712). No serious adverse event was reported.

Among patients with ARD on MTX treatment, those who withdrew the drug for 2 weeks (n=269) after third vaccine dose presented higher frequency of positive anti-SARS-CoV-2 S1/S2 IgG as well as higher GMT (183.5 (95% CI 142.7 to 236.0) vs 101.7 (95% CI 76.5 to 135.2), p=0.002) compared with those who maintained medication (55.8% vs 44.2%, p=0.029), with no statistically significant differences in NAb positivity (54.8% vs 45.7%, p=0.682) and NAb activity (83.9% (58.8%–95.9%) vs 79.0% (56.1%–91.3%), p=0.186). Comparison of patients who withheld MMF (n=109) and those who maintained the drug after the third dose showed no statistically significant differences in IgG (64.9% vs 35.1%, p=0.894) and NAb positivity (65.5% vs 34.5%, p=0.869).

DISCUSSION

This study provides novel evidence of a substantial increase in immune response with an additional dose administered 6 months after two doses of SARS-CoV-2 inactivated vaccine, in a large prospective controlled cohort of patients with ARD. We identified a distinct pattern of response to the third dose characterised by an expressive seroconversion of the COVID-19 seronegative ARD of 81% for IgG and 59% for NAb, with a parallel 11-fold increment of IgG GMT. For the COVID-19 seropositive ARD, the magnitude of IgG booster response reached almost a four-fold rise.

Immunocompromised individuals should receive a third dose of COVID-19 vaccine as proposed by the CDC[43] and WHO. This recommendation is in line with the 6-month immunogenicity waning observed for mRNA vaccines in healthcare workers, including a small proportion of individuals under immunosuppression,[44] and in a large ARD population immunised with CoronaVac.[14] Of note, no parallel increase in incident cases in the 6 months post vaccination was observed in this latter population, in contrast to the reported upsurge of cases in vaccinees with mRNA vaccine in Israel.[21]

The overall analysis of patients with ARD revealed that the additional dose resulted in global 93% IgG positivity and 33% rise. This finding is superior to the 47%–68% rate of positive antibodies reported for transplant recipients after mRNA additional dose[25 28 29 45] and may be explained in part by the distinct intensity of immunosuppression, with a high frequency of multiple drug therapy in transplanted population. In addition, the temporary discontinuation of MTX and MMF in more than half of patients under these therapies might also have contributed to improved immune response, as reported recently by our group in rheumatoid arthritis population.[46] The higher rates of anti-SARS-CoV-2 seropositivity in patients who withheld MMF herein were statistically not significant, probably due to a limited power for this analysis.

The specific analysis of immunogenicity to the third dose in non-responsive ARD 6 months after the second dose revealed a remarkable increase in IgG levels, suggesting that the three-dose strategy seems to be effective in recalling SARS-CoV-2 immune memory. The seroconversion rate of non-responsive transplant recipients was inferior, ranging from 25% to 49%, after a third dose,[24 29 47] reinforcing response differences among immunocompromised subgroups.[48] Alternatively, this observation may be related to other factors known to influence vaccine response.[44] In this regard, we have identified that prednisone, immunosuppressive drugs and biological therapy, mainly abatacept, belimumab and rituximab, were associated with decreased antibody production after the additional dose. Of note, we demonstrated herein that prednisone doses from 5 mg/day considerably reduced vaccine immune response. These same factors negatively influenced response to the primary immunisation with CoronaVac in anti-SARS-CoV-2-negative patients with ARD.[10]

COVID-19 seropositive patients with ARD had a distinct pattern of response to the third dose, with a lower but still significant increase in IgG levels, with a fourfold increase, and one-third reached the assay ceiling value (>400 AU/mL). The same

Table 4 Baseline characteristics of patients with ARD seropositive and seronegative for anti-SARS-CoV-2 S1/S2 IgG and NAb, 30 days after the third Sinovac-CoronaVac dose (D240)

	Anti-SARS-CoV-2 S1/S2 IgG			NAb		
	Seropositive (n=800)	Seronegative (n=75)	P value	Seropositive (n=683)	Seronegative (n=192)	P value
Demographic data						
Current age, years	52 (41–61)	54 (46–63)	**0.047**	52 (41–61)	54 (43.5–63)	**0.039**
Current age ≥60 years	240 (30)	28 (37.3)	0.188	202 (29.6)	66 (34.4)	0.202
Female sex	604 (75.5)	62 (82.7)	0.164	505 (73.9)	161 (83.9)	**0.004**
Caucasian race	405 (50.6)	47 (62.7)	**0.046**	351 (51.4)	101 (52.6)	0.766
ARD						
RA	253 (31.6)	29 (38.7)	0.212	191 (28.0)	91 (47.4)	**<0.0001**
SpA	100 (12.5)	4 (5.3)	0.090	93 (13.6)	11 (5.7)	**0.003**
PsA	78 (9.8)	6 (8.0)	0.623	72 (10.5)	12 (6.2)	0.075
SLE	184 (23.0)	16 (21.3)	0.742	160 (23.4)	40 (20.8)	0.450
Systemic vasculitis	44 (5.5)	11 (14.7)	**0.0018**	42 (6.1)	13 (6.9)	0.754
IIM	36 (4.5)	5 (6.7)	0.396	32 (4.7)	9 (4.7)	0.999
SSc	36 (4.5)	2 (2.7)	0.765	35 (5.1)	3 (1.6)	**0.028**
SS	36 (4.5)	1 (1.3)	0.360	30 (4.4)	7 (3.6)	0.650
PAPS	32 (4.0)	1 (1.3)	0.351	27 (4.0)	6 (3.1)	0.595
Current therapies						
Hydroxychloroquine	228 (28.5)	14 (18.7)	0.069	193 (28.3)	49 (25.5)	0.454
Sulfasalazine	74 (9.2)	3 (4.0)	0.140	64 (9.4)	13 (6.8)	0.261
Prednisone	273 (34.2)	45 (60.0)	**<0.0001**	212 (31.1)	106 (55.2)	**<0.0001**
Prednisone dose	5 (5–10)	7.5 (5–10)	0.129	5 (5–10)	5 (5–10)	0.223
Prednisone ≥5 mg/day	237 (29.6)	38 (50.7)	0.0002	186 (27.2)	89 (46.4)	<0.0001
Immunosuppressive drugs	500 (62.5)	63 (84.0)	**<0.0001**	421 (61.6)	142 (74.0)	**0.002**
Methotrexate	240 (30.0)	29 (38.7)	0.120	199 (29.1)	70 (36.5)	0.052
Leflunomide	115 (14.4)	8 (10.7)	0.377	93 (13.6)	30 (15.6)	0.479
Mycophenolate mofetil	94 (11.8)	15 (20.0)	**0.039**	87 (12.7)	22 (11.5)	0.635
Azathioprine	72 (9.0)	11 (14.7)	0.109	58 (8.5)	25 (13.0)	0.058
Tofacitinib	21 (2.6)	0 (0)	0.246	16 (2.3)	5 (2.6)	0.834
Biologic drug	257 (32.1)	45 (60.0)	**<0.0001**	202 (29.6)	100 (52.1)	**<0.0001**
Anti-TNF	119 (14.9)	13 (17.3)	0.570	99 (14.5)	33 (17.2)	0.357
Abatacept	39 (4.9)	10 (13.3)	**0.002**	23 (3.4)	26 (13.5)	**<0.0001**
Tocilizumab	37 (4.6)	5 (6.7)	0.429	30 (4.4)	12 (6.2)	0.287
Secukinumab	30 (3.8)	0 (0)	0.101	29 (4.2)	1 (0.5)	**0.011**
Belimumab	22 (2.8)	6 (8.0)	**0.014**	15 (2.2)	13 (6.8)	**0.001**
Rituximab	9 (1.1)	11 (14.7)	**<0.0001**	6 (0.9)	14 (7.3)	**<0.0001**

Results are expressed in mean±SD, median (IQR) and n (%).
Seropositivity (IgG titre ≥15 AU/mL) for anti-SARS-CoV-2 S1/S2 IgG antibodies after vaccination (Indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy). Positivity for NAb was defined as a neutralising activity ≥30% (cPass sVNT Kit, GenScript, Piscataway, USA).
p values <0.05 are highlighted in bold.

ARD, autoimmune rheumatic diseases; IIM, idiopathic inflammatory myopathy; NAb, neutralising antibodies; PAPS, primary antiphospholipid syndrome; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SpA, spondyloarthritis; SS, Sjögren's syndrome; SSc, systemic sclerosis; TNF, tumour necrosis factor.

was reported for solid organ transplant recipients and dialysis patients.[23 25 27 29] There are scarce data on NAb after third shot in immunocompromised individuals.[28 45 49 50] The overall NAb positivity in ARD reached 81% after the third dose with high median activity. These figures are similar to those reported for transplant recipients with mRNA vaccine.[28 45] We further demonstrated different immunogenicity in primary non-responsive and responsive patients with ARD. The former group achieved 59% positivity and a moderate activity, whereas COVID-19 seropositive patients with ARD had a robust response for both NAb positivity and activity. Robert *et al*[49] observed 66% response to the third dose in nine haemodialysis patients. This finding suggests that the additional dose is efficient in boosting the immune response in both ARD groups. In the context of the reported immunity waning observed for all SARS-CoV-2 vaccines at 6 months for the general population and patients with ARD,[15 44] our findings strengthen the relevance of the third dose in ARD.

The large number of patients with ARD and the inclusion of an age-balanced and sex-balanced CG are relevant strengths of the present study and provided a sizeable sample to evaluate humoral response to additional vaccine dose and the impact of the drugs. In fact, the few studies focusing on the third dose for immunocompromised individuals were small-sized[17 50] or did not have a control group.[24 25 28–30 44 46]

This study has some limitations, such as the non-assessment of cellular immunity, which may play an important role of protection during SARS-CoV-2 infection. However, this limitation was partially mitigated by NAb assessed herein, which is

Table 5 Anti-SARS-CoV-2 S1/S2 IgG and NAb seropositivity rates at baseline (D210) and after the third dose of Sinovac-CoronaVac (D240) in SARS-CoV-2 seronegative patients with ARD (ARD–), SARS-CoV-2 seropositive ARD (ARD+), SARS-CoV-2 seronegative controls (CG–) and SARS-CoV-2 seropositive controls (CG+)

	Frequency of anti-S1/S2 seropositivity, n (%)			Anti-S1/S2 IgG GMT (95% CI), AU/mL			Frequency of NAb positivity, n (%)			Percentage of NAb activity, median (IQR)		
Groups	D210	D240	P value	D210	D240	P value	D210	D240	P value	D210	D240	P value
ARD– (n=215)	0 (0)	173 (80.5)	**<0.0001**	5.0 (4.6 to 5.6)	56.9 (46.1 to 70.3)	**<0.001**	0 (0)	127 (59.1)	**<0.0001**	–	–	
CG– (n=43)	0 (0)	43 (100)	**<0.0001**	8.4 (7.1 to 9.9)	200.0 (166.3 to 240.5)	**<0.001**	0 (0)	42 (97.7)	**<0.0001**	–	–	
P value (ARD– vs CG–)	>0.999	**0.0004**		**<0.001**	**<0.001**		>0.999	**<0.0001**				
ARD+ (n=316)	307 (97.2)	316 (100)	0.0077	65.7 (58.3 to 73.9)	255.2 (231.1 to 281.8)	**<0.001**	196 (62.0)	291 (92.1)	**<0.0001**	64.4 (44.8–91.5)	88.7 (70.2–96.7)	**<0.001**
CG+ (n=158)	157 (99.4)	158 (100)	>0.999	76.3 (65.6 to 88.8)	264.7 (246.0 to 284.9)	**<0.001**	106 (67.1)	156 (98.7)	**<0.0001**	77.1 (59.9–93.9)	93.2 (82–96.7)	**<0.001**
P value (ARD+ vs CG+)	0.176	>0.999		0.128	0.567		0.280	**0.0025**		**0.022**	**0.025**	

Frequencies of subjects with positive anti-SARS-CoV-2 S1/S2 IgG and NAb are expressed as number (%).
Positivity for anti-SARS-CoV-2 S1/S2 IgG was defined as postvaccination titre ≥15 AU/mL by Indirect ELISA (LIAISON SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy). Positivity for NAb was defined as a neutralising activity ≥30% (cPass sVNT Kit, GenScript, Piscataway, USA).
Frequencies of seropositivity were compared using McNemar's test for before and after comparisons and χ^2 or Fisher's exact tests for ARD vs CG comparisons.
Percentages of neutralising activity of NAb are expressed as median (IQR) and anti-S1/S2 IgG antibody titres are expressed as geometric mean with 95% CI. The ln-transformed IgG titres and NAb activity were compared between groups (ARD and CG) and timepoints (D210 and D240) using repeated measures analysis of variance with two factors followed by Bonferroni's multiple comparisons in Napierian ln-transformed data.
p values <0.05 are highlighted in bold.
ARD, autoimmune rheumatic diseases; CG, control group; D210, 6 months after second dose; D240, 30 days after third dose; GMT, geometric mean titres; ln, logarithm; NAb, neutralising antibodies.

highly predictive of immune protection.[51] The assessment of the effectiveness of the third dose was hampered by the short-term follow-up and the very low incidence of COVID-19 cases during the study period. The global analysis of the influence of some drugs that are used only for specific ARD in the immunogenicity evaluation performed herein probably underestimated the effect of these medications. However, these therapies remained as relevant independent factors that negatively impacted immunogenicity in the multivariate evaluation. The lack of disease activity assessment, especially for those who withdrew MTX or MMF, is also an important limitation.

In summary, to our knowledge this is the first demonstration of a robust response to the third dose of an inactivated vaccine in patients with ARD, with greater benefit for those who are COVID-19 seronegative before the third dose. We further identified drugs as unfavourable for response to additional vaccine doses. These findings may be generalised to other platform vaccines, and heterologous fourth-dose boosting could be an alternative strategy for the minority of persistently non-responsive patients with ARD.

Author affiliations
[1]Rheumatology Division, Hospital das Clinicas, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil
[2]Infectious Disease Department, Hospital das Clinicas, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil
[3]Central Laboratory Division, Hospital das Clinicas, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil
[4]Pediatric Rheumatology Unit, Instituto da Criança e do Adolescente, Hospital das Clinicas, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil

Acknowledgements We thank the volunteers for participating in all inperson visits and for handling the biological material, as well as those responsible for the follow-up of all participants. We also thank the contribution of the undergraduate and postgraduate students for collecting epidemiological and clinical data, as well as the Central Laboratory Division, Registry Division, Security Division, IT Division, Superintendence, Pharmacy Division and Vaccination Center for their technical support. Instituto Butantan supplied the study product and had no other role in the trial.

Contributors NEA, CAS, LdVKK, ACM-R, CGSS, EFNY, SGP and EB conceived and designed the study, participated in data collection and analysis, supervised the clinical data management, and wrote and revised the manuscript. EB is responsible for the overall content as the guarantor. EB and PR organised and supervised the blood collection and vaccination. SGP, VAOM and VLNB supervised the serum processing, SARS-CoV-2-specific antibody ELISA/neutralisation assays and SARS-CoV-2 RT-PCR. NEA, LdVKK, ACM-R, CGSS, EFNY, SGP, PTR, RMRP, SKS, PDS-B, DA, ASRH, RF, FHCS, LKNG, APLA, JCBdM, MRUL, VAOM, LB, CTR, LPS, IMB, RLPM, GGMB, LA, AMCS, CAS and EB collected epidemiological and clinical data and assisted with the identification of SARS-CoV-2 infection and follow-up of patients. PR organised and supervised the vaccination protocol. All authors helped to edit the manuscript.

Funding This study was sponsored by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (#2015/03756-4 to CAS, SGP, NEA and EB; #2019/17272-0 to LdVKK; #2020/09367-8; #2018/09937-9 to VAOM), Conselho Nacional de Desenvolvimento Científico e Tecnológico (#304984/2020-5 to CAS; #305556/2017-7 to RMRP; #303379/2018-9 to SKS; #305242/2019-9 to EB), B3-Bolsa de Valores do Brasil and Chamada Instituto Todos pela Saúde (ITPS 01/2021, proposta C1313 to EB, CAS, NEA and SGP).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study involves human participants. The protocol was approved by the national and institutional ethical committee (Conep, Comissão Nacional De Ética Em Pesquisa; reference number: CAAE: 42566621.0.0000.0068). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article.

ORCID iDs
Nádia Emi Aikawa http://orcid.org/0000-0002-7585-4348
Danieli Castro Oliveira Andrade http://orcid.org/0000-0002-0381-1808
Eloisa Bonfa http://orcid.org/0000-0002-0520-4681

REFERENCES

1 Jara A, Undurraga EA, González C, *et al*. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. *N Engl J Med Overseas Ed* 2021;385:875–84.
2 Subramanian SV, Kumar A. Increases in COVID-19 are unrelated to levels of vaccination across 68 *countries* and 2947 counties in the United States. *Eur J Epidemiol* 2021;36:1237-1240.
3 Brown CM, Vostok J, Johnson H, *et al*. Outbreak of SARS-CoV-2 infections, including COVID-19 vaccine breakthrough infections, associated with large public Gatherings — Barnstable County, Massachusetts, July 2021. *MMWR Morb Mortal Wkly Rep* 2021;70:1059–62.
4 Dougherty K, Mannell M, Naqvi O, *et al*. SARS-CoV-2 B.1.617.2 (Delta) Variant COVID-19 Outbreak Associated with a Gymnastics Facility - Oklahoma, April-May 2021. *MMWR Morb Mortal Wkly Rep* 2021;70:1004-1007.

5 Shitrit P, Zuckerman NS, Mor O, *et al*. Nosocomial outbreak caused by the SARS-CoV-2 delta variant in a highly vaccinated population, Israel, July 2021. *Eurosurveillance* 2021;26:26.
6 Chau NVV, Ngoc NM, Nguyet LA, *et al*. An observational study of breakthrough SARS-CoV-2 delta variant infections among vaccinated healthcare workers in Vietnam. *EClinicalMedicine* 2021;41:101143.
7 Rosenberg ES, Holtgrave DR, Dorabawila V. New COVID-19 cases and hospitalizations among adults, by vaccination status — New York, may 3–July 25, 2021. *MMWR Morb Mortal Wkly Rep* 2021:70.
8 Mbaeyi S, Oliver SE, Collins JP. Morbidity and Mortality Weekly Report The Advisory Committee on Immunization Practices' Interim Recommendations for Additional Primary and Booster Doses of COVID-19 Vaccines-United States, 2021, 2021. Available: https://www.cdc.gov/vaccines/hcp/acip-recs/vacc-specific/covid-19.html
9 Shapiro Ben David S, Shamir-Stein Na'ama, Baruch Gez S, *et al*. Reactogenicity of a third BNT162b2 mRNA COVID-19 vaccine among immunocompromised individuals and seniors - A nationwide survey. *Clin Immunol* 2021;232:108860.
10 Medeiros-Ribeiro AC, Aikawa NE, Saad CGS, *et al*. Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial. *Nat Med* : 2021;27:1744-1751.
11 Pablos JL, Galindo M, Carmona L, *et al*. Clinical outcomes of hospitalised patients with COVID-19 and chronic inflammatory and autoimmune rheumatic diseases: a multicentric matched cohort study. *Ann Rheum Dis* 2020;79:1544-1549.
12 Bertoglio IM, Valim JMdeL, Daffre D, *et al*. Poor Prognosis of COVID-19 Acute Respiratory Distress Syndrome in Lupus Erythematosus: Nationwide Cross-Sectional Population Study Of 252 119 Patients. *ACR Open Rheumatol* 2021;3. doi:10.1002/acr2.11329. [Epub ahead of print: Published Online First].
13 Aikawa NE, Kupa LVK, Pasoto SG, *et al*. Immunogenicity and safety of two doses of the CoronaVac SARS-CoV-2 vaccine in SARS-CoV-2 seropositive and seronegative patients with autoimmune rheumatic diseases in Brazil: a subgroup analysis of a phase 4 prospective study. *Lancet Rheumatol* 2022;4:e113-e124.
14 Chia WN, Zhu F, Ong SWX, *et al*. Dynamics of SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study. *Lancet Microbe* 2021;2:e240–9.
15 Silva CA, Medeiros-Ribeiro AC, Kupa LVK. Anti-SARS-CoV-2 immunogenicity decay and incident cases six months after Sinovac-CoronaVac inactivated vaccine in autoimmune rheumatic disease patients: phase 4 prospective trial, 23 November 2021, PREPRINT (version 1) available at research square.
16 Yue L, Zhou J, Zhou Y, *et al*. Antibody response elicited by a third boost dose of inactivated SARS-CoV-2 vaccine can neutralize SARS-CoV-2 variants of concern. *Emerg Microbes Infect* 2021;10:2125–7.
17 Schmiedeberg K, Vuilleumier N, Pagano S, *et al*. Efficacy and tolerability of a third dose of an mRNA anti-SARS-CoV-2 vaccine in patients with rheumatoid arthritis with absent or minimal serological response to two previous doses. *Lancet Rheumatol* 2022;4:e11–13.
18 Karaba AH, Zhu X, Liang T, *et al*. A third dose of SARS-CoV-2 vaccine increases neutralizing antibodies against variants of concern in solid organ transplant recipients. *medRxiv* 2021:2021.08.11.21261914.
19 Bar-On YM, Goldberg Y, Mandel M, *et al*. Protection of BNT162b2 vaccine booster against Covid-19 in Israel. *N Engl J Med* 2021;385:1393–400.
20 Zeng G, Wu Q, Pan H, *et al*. Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials. *Lancet Infect Dis* 2021;7:S1473-3099(21)00681-2. doi:10.1016/S1473-3099(21)00681-2. [Epub ahead of print: 07 Dec 2021].
21 Barda N, Dagan N, Cohen C, *et al*. Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for preventing severe outcomes in Israel: an observational study. *Lancet* 2021;398:2093–100.
22 Flaxman A, Marchevsky NG, Jenkin D, *et al*. Reactogenicity and immunogenicity after a late second dose or a third dose of ChAdOx1 nCoV-19 in the UK: a substudy of two randomised controlled trials (COV001 and COV002). *Lancet* 2021;398:981–90.
23 Bensouna I, Caudwell V, Kubab S, *et al*. SARS-CoV-2 antibody response after a third dose of the BNT162b2 vaccine in patients receiving maintenance hemodialysis or peritoneal dialysis. *Am J Kidney Dis* 2022;79:185–92.
24 Benotmane I, Gautier G, Perrin P, *et al*. Antibody response after a third dose of the mRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients with minimal serologic response to 2 doses. *JAMA* 2021;326:1063–5.
25 Bertrand D, Hamzaoui M, Lemée V, *et al*. Antibody and T-cell response to a third dose of SARS-CoV-2 mRNA BNT162b2 vaccine in kidney transplant recipients. *Kidney Int* 2021;100:1337–40.
26 Le Bourgeois A, Coste-Burel M, Guillaume T, *et al*. Interest of a third dose of BNT162b2 anti-SARS-CoV-2 messenger RNA vaccine after allotransplant. *Br J Haematol* 2022;196:e38-e40.
27 Espi M, Charmetant X, Barba T. Justification, safety, and efficacy of a third dose of mRNA vaccine in maintenance hemodialysis patients: a prospective observational study list of collaborators from the REIN registry 2021.
28 Hall VG, Ferreira VH, Ku T, *et al*. Randomized trial of a third dose of mRNA-1273 vaccine in transplant recipients. *N Engl J Med* 2021;385:1244–6.
29 Kamar N, Abravanel F, Marion O, *et al*. Three doses of an mRNA Covid-19 vaccine in solid-organ transplant recipients. *N Engl J Med* 2021;385:661–2.
30 Marlet J, Gatault P, Maakaroun Z, *et al*. Antibody responses after a third dose of covid-19 vaccine in kidney transplant recipients and patients treated for chronic lymphocytic leukemia. *Vaccines* 2021;9. doi:10.3390/vaccines9101055. [Epub ahead of print: 23 09 2021].
31 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against rheumatism collaborative initiative. *Arthritis Rheum* 2010;62:2569–81.
32 Petri M, Orbai A-M, Alarcón GS, *et al*. Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012;64:2677–86.
33 Rudwaleit M, van der Heijde D, Landewé R, *et al*. The development of assessment of spondyloarthritis International Society classification criteria for axial spondyloarthritis (Part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.
34 Tillett W, Costa L, Jadon D, *et al*. The CIASsification for Psoriatic ARthritis (CASPAR) criteria--a retrospective feasibility, sensitivity, and specificity study. *J Rheumatol* 2012;39:154–6.
35 Arend WP, Michel BA, Bloch DA, *et al*. The American College of rheumatology 1990 criteria for the classification of Takayasu arteritis. *Arthritis & Rheumatism* 1990;33:1129–34.
36 Leavitt RY, Fauci AS, Bloch DA. The American College of Rheumatology 1990 criteria for the classification of wegener's granulomatosis. *Arthritis Rheum* 1990;33.
37 Vitali C, Bombardieri S, Jonsson R, *et al*. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European consensus group. *Ann Rheum Dis* 2002;61:554–8.
38 van den Hoogen F, Khanna D, Fransen J, *et al*. 2013 classification criteria for systemic sclerosis: an American College of rheumatology/European League against rheumatism collaborative initiative. *Ann Rheum Dis* 2013;72:1747–55.
39 Lundberg IE, Tjärnlund A, Bottai M. EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann Rheum Dis* 2018;76:1955–64.
40 Miyakis S, Lockshin MD, Atsumi T, *et al*. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006;4:295-306.
41 Taylor SC, Hurst B, Charlton CL, *et al*. A new SARS-CoV-2 dual-purpose serology test: highly accurate infection tracing and neutralizing antibody response detection. *J Clin Microbiol* 2021;59. doi:10.1128/JCM.02438-20. [Epub ahead of print: 19 03 2021].
42 Curtis JR, Johnson SR, Anthony DD. American College of rheumatology guidance for COVID-19 vaccination in patients with rheumatic and musculoskeletal diseases – version 3. *Arthritis Rheumatol* 2021. doi:10.1002/art.41928
43 COVID-19 vaccine booster Shots. Available: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/booster-shot.html#choosing-booster
44 Levin EG, Lustig Y, Cohen C, *et al*. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. *N Engl J Med* : 2021;385:e84.
45 Peled Y, Ram E, Lavee J, *et al*. Third dose of the BNT162b2 vaccine in heart transplant recipients: immunogenicity and clinical experience. *J Heart Lung Transplant* 2022;41:02481–5.
46 Araujo CSR, Medeiros-Ribeiro AC, Saad CGS, *et al*. Two-Week methotrexate discontinuation in patients with rheumatoid arthritis vaccinated with inactivated SARS-CoV-2 vaccine: a randomised clinical trial. *Ann Rheum Dis* 2022;0:1–9.
47 Werbel WA, Boyarsky BJ, Ou MT, *et al*. Safety and immunogenicity of a third dose of SARS-CoV-2 vaccine in solid organ transplant recipients: a case series. *Ann Intern Med* 2021;174:1330–2.
48 Embi PJ, Levy ME, Naleway AL, *et al*. Effectiveness of 2-Dose Vaccination with mRNA COVID-19 Vaccines Against COVID-19-Associated Hospitalizations Among Immunocompromised Adults - Nine States, January-September 2021. *MMWR Morb Mortal Wkly Rep* 2021;70:1553–9.
49 Robert T, Lano G, Giot M, *et al*. Humoral response after SARS-CoV-2 vaccination in patients undergoing maintenance haemodialysis: loss of immunity, third dose and non-responders. *Nephrol Dial Transplant* 2022;37:390–2.
50 Shroff RT, Chalasani P, Wei R, *et al*. Immune responses to two and three doses of the BNT162b2 mRNA vaccine in adults with solid tumors. *Nat Med* 2021;27:2002–11.
51 Khoury DS, Cromer D, Reynaldi A, *et al*. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021;27:1205–11.

4.4. CoronaVac induz maior produção de anticorpos em pessoas com hepatite B crônica, diz estudo chinês

Um estudo publicado na revista Liver International mostrou que a CoronaVac, vacina do Butantan e da Sinovac, é altamente eficaz em pacientes com hepatite B crônica, induzindo maior produção de anticorpos nesse público do que em indivíduos saudáveis. O trabalho foi conduzido por cientistas da Universidade Médica de Chongqing, na China. A hepatite crônica é uma inflamação do fígado causada pelo vírus da hepatite B que dura mais de seis meses e aumenta o risco de ter câncer hepático.

Participaram do estudo 362 pacientes adultos com hepatite crônica (sendo 48 com cirrose e 73 com replicação viral ativa) e 87 indivíduos saudáveis no grupo controle, que foram vacinados com duas doses de vacinas de vírus inativados, a CoronaVac ou o imunizante da Sinopharm.

A taxa de soroconversão de anticorpos IgG foi similar entre os dois grupos após um, dois e três meses da segunda dose. Já o título de anticorpos foi menor nos pacientes no primeiro mês, mas a partir do segundo esse valor se equiparou ao de indivíduos saudáveis. "Curiosamente, no terceiro mês, os pacientes apresentaram títulos de anticorpos maiores do que os controles", afirmam os autores do artigo.

Os pesquisadores avaliaram os títulos de anticorpos IgG anti-Spike, anti-RBD e bloqueadores de RBD--ACE2. Ao comparar pacientes e controles, os resultados mostraram titulação de 161,6 vs. 85,9; 275,4 vs. 132,5 e 4,7 vs. 3,2, respectivamente.

Além disso, os voluntários com hepatite crônica apresentaram um declínio mais lento de anticorpos ao longo do tempo do que as pes-

soas sem a doença. Também não houve diferença na resposta imune entre pacientes com e sem cirrose e com e sem replicação viral ativa.

Todos os efeitos adversos relatados foram leves e a incidência de reações foi semelhante entre pacientes e controles (14% e 11,5%, respectivamente). Os sintomas mais comuns foram dor no local da injeção e fadiga.

CoronaVac é segura para pacientes com doenças no fígado

Uma série de estudos já atestou a segurança e eficácia da vacina do Butantan para pessoas com problemas no fígado. Uma pesquisa chinesa mostrou que a vacina induziu altas taxas de soroconversão em pacientes com hepatite B, sendo 87,25% para anticorpos IgG e 74,5% para os anticorpos neutralizantes. Outro trabalho publicado na plataforma de preprints SSRN, da The Lancet, demonstrou 100% de produção de anticorpos IgG em indivíduos com doença hepática gordurosa associada ao metabolismo (DHGM).

Publicado em: 02/02/2022

He Taiyu (Orcid ID: 0000-0002-4420-6364)
Hu Peng (Orcid ID: 0000-0001-8481-0841)

Safety and antibody response to inactivated COVID-19 vaccine in patients with chronic hepatitis B virus infection

Taiyu He[#], Yingzhi Zhou[#], Pan Xu[#], Ning Ling[#], Min Chen[#], Tianquan Huang, Biqiong Zhang, Ziqiao Yang, Ling Ao, Hu Li, Zhiwei Chen, Dazhi Zhang, Xiaofeng Shi, Yu Lei, Zhiyi Wang, Weiqun Zeng, Peng Hu, Yinghua Lan, Zhi Zhou, Juan Kang, Ying Huang, Tongdong Shi, Qingbo Pan, Qian Zhu, Xiping Ran, Yingzhi Zhang, Rui Song, Dejuan Xiang, Shuang Xiao, Gaoli Zhang, Wei Shen, Mingli Peng[*], Dachuan Cai[*], Hong Ren[*]

Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Institute for Viral Hepatitis, Department of Infectious Diseases, the Second Affiliated Hospital, Chongqing Medical University, Chongqing, China

* Corresponding authors: Mingli Peng[*], Dachuan Cai[*], Hong Ren[*]

Address: Institute for Viral Hepatitis, Chongqing Medical University, Chongqing, 400000, China; E-mail addresses: peng_mingli@hospital.cqmu.edu.cn (Mingli Peng), cqmucdc@cqmu.edu.cn (Dachuan Cai), renhong0531@vip.sina.com (Hong Ren).

[#] Co-first authors: Taiyu He[#], Yingzhi Zhou[#], Pan Xu[#], Ning Ling[#], Min Chen[#]

ORCID: https://orcid.org/0000-0002-4420-6364 (Taiyu He)

Word count (main body of manuscript): 4537

Table: 3; **Figure:** 2; **Supplementary Table:** 8; **Supplementary Figure:** 5

Abbreviations:

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/liv.15173

ACE, angiotensin-converting enzyme; AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CHB, chronic hepatitis B; CLD, chronic liver disease; COVID-19, coronavirus disease 2019; FBS, fetal bovine serum; GMTs, Geometric mean titers; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; IHCs, inactive HBsAg carriers; NAFLD, non-alcoholic fatty liver disease; PBMCs, peripheral blood mononuclear cells; PC, plasma cell; PLT, platelet; RBD, receptor binding domain; SAEs, serious adverse events; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; TB, total bilirubin; WBC, white blood cell.

Conflict of interest: The authors declare no conflicts of interest.

Funding: This work is supported by the National Science and Technology Major Project of China (2017ZX10202203-007, 2017ZX10202203-008, 2018ZX10302206-003) and a pilot project of clinical cooperation between traditional Chinese and western medicine for significant and complicated diseases of National Administration of Traditional Chinese Medicine: hepatic fibrosis. We also acknowledge the support of the National Natural Science Foundation of China (81772198), Natural Science Foundation of Chongqing, China (cstc2020jcyj-msxmX0389).

Trial registration number: ChiCTR2100047936 and NCT05007665.

Acknowledgement: We thank the health care center and department of clinical laboratory of the Second Affiliated Hospital, Chongqing Medical University for their support.

Author contributions:

Concept and design: Hong Ren, Dachuan Cai, Mingli Peng

Funding acquisition: Hong Ren, Peng Hu, Mingli Peng, Min Chen

Participant recruitment and characterisation: Ning Ling, Dachuan Cai, Tianquan Huang, Biqiong Zhang, Ziqiao Yang, Dazhi Zhang, Xiaofeng Shi, Yu Lei, Zhiyi Wang, Weiqun Zeng, Peng Hu, Yinghua Lan, Zhi Zhou, Juan Kang, Ying Huang, Tongdong Shi, Qingbo Pan, Qian Zhu, Xiping Ran, Rui Song, Taiyu He, Pan Xu

Experiment execution: Yingzhi Zhou, Taiyu He, Ling Ao, Yingzhi Zhang, Dejuan Xiang, Shuang Xiao, Gaoli Zhang, Ming Chen, Mingli Peng

Acquisition, analysis or interpretation of data: Taiyu He, Yingzhi Zhou, Pan Xu, Ning Ling, Min Chen, Tianquan Huang, Biqiong Zhang, Ziqiao Yang, Hu Li, Zhiwei Chen, Wei Shen, Mingli Peng, Dachuan Cai, Hong Ren

Supervision: Hong Ren

Administrative support: Hong Ren, Dachuan Cai, Mingli Peng

Drafting and critical revision of manuscript: Taiyu He, Zhiwei Chen, Hu Li, Ning Ling, Min Chen, Mingli Peng, Dachuan Cai, Hong Ren

All authors contributed to the article and approved the submitted version.

Data availability statement: The authors declare that all data included in this article will be made available upon request.

Abstract:

Background & Aims: The safety and antibody responses of coronavirus disease 2019 (COVID-19) vaccination in patients with chronic hepatitis B (CHB) virus infection is still unclear, and exploration in safety and antibody responses of COVID-19 vaccination in CHB patients is significant in clinical practice.

Methods: 362 adult CHB patients and 87 healthy controls at an interval of at least 21 days after full-course vaccination (21-105 days) were enrolled. Adverse events (AEs) were collected by questionnaire. The antibody profiles at 1, 2 and 3 months were elucidated by determination of anti-spike IgG, anti-receptor binding domain (RBD) IgG, and RBD-angiotensin-converting enzyme 2 blocking antibody. SARS-CoV-2 specific B cells were also analyzed.

Results: All AEs were mild and self-limiting, and the incidence was similar between CHB patients and controls. Seropositivity rates of three antibodies were similar between CHB patients and healthy controls at 1, 2 and 3 months, but CHB patients had lower titers of three antibodies at 1 month. Compared to healthy controls, HBeAg-positive CHB patients had higher titers of three antibodies at 3 month (all $p < 0.05$) and a slower decline in antibody titers. Frequency of RBD-specific B cells was positively correlated with titers of anti-RBD IgG (OR=1.067, p=0.004), while liver cirrhosis, antiviral treatment, levels of HBV DNA, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and total bilirubin (TB) were not correlated with titers of anti-RBD IgG.

Conclusions: Inactivated COVID-19 vaccines were well tolerated, and induced

effective antibody response against SARS-CoV-2 in CHB patients.

Word count of the abstract: 244

Keywords: COVID-19 vaccine; safety; antibody response; CHB; cirrhosis

Lay Summary: COVID-19 vaccines are safe for CHB patients. After full-course of inactivated COVID-19 vaccination, the proportion of CHB patients who produced antibody was similar to that of healthy people who produced antibody. The antibody levels are lower in CHB patients at 1 month after full-course of vaccination.

Introduction

COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged as a major burden worldwide, resulting in serious public health challenges. Patients with liver diseases may have a greater risk of worse outcome from COVID-19 than the general population[1-3]. Currently, there are about 292 million people worldwide infected with HBV, and 86 million in China alone[4].

Vaccination is an effective intervention in preventing SARS-CoV-2 infection, severe symptom and death[5-7]. Recently, there are several studies on safety and immunogenicity of COVID-19 vaccination in patients with liver disease. Wang et al.[8] reported that inactivated COVID-19 vaccine appeared to be safe with good immunogenicity in patients with non-alcoholic fatty liver disease (NAFLD). Thuluvath et al.[9] showed that 24% of those with chronic liver disease (CLD) had poor antibody response 4 weeks after SARS-CoV-2 vaccination. Darius et al.[10] found normal humoral response and poor T-cell response in 53 cirrhotic patients. CHB patients have dysregulated innate and adaptive immunity[11-13]. But until now, whether immunity-dysregulated CHB patients can be safely inoculated with COVID-19 vaccine and produce antibody response similar to that of healthy people are still unclear. Moreover, in clinical practice, CHB patients, especially those with cirrhosis, often have concerns about safety and efficacy of COVID-19 vaccination in them, and clinicians lack corresponding evidence from clinical research to respond to these patients' concerns.

Inactivated vaccine is a type of COVID-19 vaccine which is widely used in China and other countries around the world. This study aimed to investigate the safety and

antibody responses (spike-specific IgG, RBD-specific IgG, and RBD-angiotensin-converting enzyme 2 (ACE2) blocking antibody) to COVID-19 inactivated vaccines in CHB patients at 1, 2 and 3 months after full-course vaccination. Furthermore, RBD-specific B cell response was detected to explore the mechanism of antibody response.

Methods

Participants

362 adult CHB patients (including 48 cirrhotic patients) and 87 adult healthy individuals were enrolled in this cross-sectional observational study between 1 July 2021 and 27 August 2021 at the Second Affiliated Hospital of Chongqing Medical University. The inclusion criteria for patients were hepatitis B surface antigen (HBsAg) positive more than six months, and diagnosis of liver cirrhosis was made based on guideline[14]. The inclusion criteria for healthy controls were HBsAg negative, with no self-reported and documented disease status. For all participants, the following conditions were excluded: a) a history of COVID-19 hospitalization; b) a positive result of SARS-CoV-2 nucleic acid test (all participants had taken the test at least once since COVID-19 pandemic); c) close contact with SARS-CoV-2 confirmed cases; d) COVID-19 symptoms such as fever, cough, fatigue, etc. during the pandemic (adverse events after vaccination were excepted).; e) a travel history or residence history to Wuhan during the COVID-19 outbreak; f) coinfection with HIV/HCV; g) malignant tumor (including liver cancer), renal failure, and other major diseases; h) pregnancy; i) use of immunosuppressant. All participants completed full-course vaccination (two

doses) of SARS-CoV-2 inactivated vaccine (BBIBP-CorV/CoronaVac). This study was approved by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University and conformed with the ethical guidelines of the Declaration of Helsinki. This study has been registered at www.chictr.org.cn (ChiCTR2100047936) and ClinicalTrials.gov (NCT05007665), and the follow-up is still going on. Written informed consent was obtained from all participants prior to their inclusion in the study.

Data collection

Demographic and clinical data were obtained by questionnaire and electronic medical record, and their peripheral blood was sampled at an interval of at least 21 days (21-105 days) after the full-course vaccination for the detection of SASR-CoV-2 spike-specific IgG, RBD-specific IgG, RBD-ACE2 blocking antibody and RBD-specific B cells. Considering the interval after full-course vaccination, we defined the gap of 21-45 days as “1 month”, 46-75 days as “2 month”, and 76-105 days as “3 month”. There was only one sample per patient that matched one of the study times. In total, 210 participants were in the “1 month” group, 141 participants were in the “2 month” group, and 98 participants were in the “3 month” group.

Safety and antibody response assessment

The overall incidence of adverse events within 7 days and 30 days was compared between CHB patients and healthy controls to assess safety. Titers of three antibodies, including anti-spike IgG, anti-RBD IgG and spike RBD-ACE2 blocking antibody, at 1, 2 and 3 months after full-course vaccination were comprehensively compared and analyzed to assess antibody response.

Adverse events monitoring

Participants' AEs were obtained by questionnaire and were verified by investigators. All AEs were graded according to the scale issued by National Medical Products Administration of China (version 2019).

Evaluation of anti-spike IgG and anti-RBD IgG

According to the manufacturer's protocol, the indirect ELISA method was used to detect IgG binding antibodies against spike and RBD protein (KIT004 and KIT002, Sino Biological, Beijing, China). Briefly, 0.5 ug/mL recombinant Spike (S1+S2) or RBD protein was pre-coated on the plate wells (100 μL per well) by incubation at 4 □ overnight. After thoroughly discarding solutions in the plate, 300 μL of 6% BSA solution was added to each well and incubated for 1 hour at room temperature. After washing wells thoroughly, serially diluted samples or controls (100 μL) were added, mixed well, and incubated for 2 hours at room temperature. Following three times of washing plates, diluted horseradish peroxidase (HRP) conjugated goat anti-human IgG secondary antibody was added (100 μL per well), mixed well, and incubated for 1 hour at room temperature. After washing and adding substrate solution (TMB) and then stop solution, absorbance (OD value) was read at 450 nm. Serum samples were diluted with two-folded serial dilution starting from 1:50. In each plate, serially diluted positive antibody controls (anti-spike or anti-RBD antibody) and negative controls (serum from individuals without a history of SARS-CoV-2 infection and vaccination) were detected simultaneously. ELISA measurements were performed in duplicate. According to the manufacturer's instructions, a serum was considered seropositive for IgG binding

antibodies when OD value ≥ 2.1 times the mean absorbance value of negative controls at 1:50 dilution. The antibody level was presented as the highest serum dilution showing a positive result.

Assay for the SARS-CoV-2 spike RBD-ACE2 blocking antibody

The SARS-CoV-2 spike RBD-ACE2 blocking antibody can compete with ACE2 to combine with RBD, and it can represent the functional (neutralizing) antibody. The level of blocking antibody was assayed by competitive ELISA according to the manufacturer's protocol (KIT001, Sino Biological, Beijing, China). Briefly, microplates were coated with 1 μg/mL human ACE2 recombinant protein (100 μL per well) by incubation at 4 ℃ overnight. After thoroughly discarding solutions in the plate, 300 μL of 6% BSA solution was added to each well and incubated for 1 hour at room temperature. After washing wells thoroughly, serially diluted samples (50 μL) and RBD protein linked to HRP (RBD-HRP) (50 μL) were added at the same time, mixed well, and incubated for 30 min at room temperature. After washing and adding substrate solution (TMB) and then stop solution, absorbance (OD value) was read at 450 nm. Serum samples were diluted with two-folded serial dilution starting from 1:5. In each plate, RBD-HRP only control, serially diluted positive controls (SARS-CoV-2 Inhibitors) and negative controls (serum from individuals without a history of SARS-CoV-2 infection and vaccination) were detected at the same time. ELISA measurements were performed in duplicate. Inhibition rate was calculated as 100 - [(OD value of sample/ OD value of RBD-HRP only control) × 100%]. According to the manufacturer's instructions, a positive result for the SARS-CoV-2 blocking antibody

was determined when the inhibition rate was ⩾ 20%. The blocking antibody level was presented as the highest serum dilution showing a positive result.

Detection of SARS-CoV-2 specific B cells by flow cytometry

For SARS-CoV-2 specific B cells detection, biotinylated SARS-CoV-2 Spike RBD protein (40592-V08H2-B, Sino Biological, Beijing, China) was mixed with Streptavidin-BV421 (405225, Biolegend, California, USA) at 4:1 molar ratio for one hour at 4℃ to obtain the antigen probe. According to manufacturer's instruction, peripheral blood mononuclear cells (PBMCs) were isolated from heparinized whole blood by Histopaque (10771, Sigma-Aldrich, Missouri, USA) density gradient centrifugation. After washed by FACS buffer (PBS＋2% FBS (FSD500, Excell Bio, Shanghai, China)), around 0.5×10^6 PBMCs were then stained for 30 minutes at 4℃ using antigen probe (1:33.3) and the following conjugated antibodies: anti-human CD3 (1:50, 300430, Biolegend, California, USA), anti-human CD19 (1:50, 302212, Biolegend), anti-human CD21 (1:50, 354918, Biolegend), anti-human CD27 (1:50, 356406, Biolegend), anti-human CD38 (1:50, 303504, Biolegend), anti-human IgG Fc (1:50, 410722, Biolegend), and anti-human IgM (1:50, 314524, Biolegend). After staining, cells were washed and resuspended in a 200ul FACS buffer. Roughly 1×10^5 events (cells) were then acquired within a lymphocyte gate on flow cytometer (CytoFLEX, Beckman Coulter, California, USA). Data analysis on RBD-specific B cell population and its subpopulations was conducted by using FlowJo (10.0.7r2, Treestar, Oregon, USA). And the cell populations were as follows: RBD-specific B cell ($CD3^-CD19^+RBD^+$), RBD-specific memory B cell (MBC) ($CD3^-CD19^+RBD^+CD27^+$), RBD^+

atypical MBC ($CD3^-CD19^+RBD^+CD21^-CD27^-$), RBD^+ activated MBC ($CD3^-CD19^+RBD^+CD21^-CD27^+$), RBD^+ resting MBC ($CD3^-CD19^+RBD^+CD21^+CD27^+$), RBD^+ intermediate MBC ($CD3^-CD19^+RBD^+CD21^+CD27^-$), RBD^+IgG^+ MBC ($CD3^-CD19^+RBD^+CD27^+IgG^+$), RBD^+IgM^+ MBC ($CD3^-CD19^+RBD^+CD27^+IgM^+$), and RBD^+CD38^+ MBC ($CD3^-CD19^+RBD^+CD27^+CD38^+$). The full gating strategy is shown in the Supplementary Figure 1.

Statistical analysis

Appropriate methods were used for statistical analysis based on the type of data. For categorical variables, Chi-Square test and Fisher's exact test were used. For continuous variables, Mann-Whitney U test was used to compare two groups, and Kruskal-Wallis test was used to compare three or more groups. All results of multiple comparisons were corrected using Bonferroni's correction or Dunn's multiple comparisons test, and p-values represented in figures were all adjusted p-values. Spearman's rank correlation was applied for correlation between antibodies titers. Univariate and multivariate ordinal logistic regression analyses were used to obtain factors that significantly affected antibody titers. Categorical variables were presented as numbers (%), and continuous variables were presented as median (Range). When the antibody titer of participant was lower than the detection limit, half value of the detection limit was assigned. A two-sided p-value<0.05 was considered statistically significant. SPSS (24.0.0, IBM, New York, USA) and Graphpad Prism (9.2.0, GraphPad Software Inc, California, USA) were used for statistical analysis. Graphpad Prism was used for plotting.

Results

1. Characteristics of participants

As shown in Table 1, the median age (45 vs. 44, p=0.143) and median body mass index (BMI) (23.6 vs. 23.4, p=0.752) were similar between CHB patients and healthy controls. More than half of patients (61.6%, 223/362) and controls (50.6%, 44/87) were male (p=0.060). Compared to controls, CHB patients had lower levels of white blood cell (WBC) (5.37 vs. 5.70, p=0.039) and platelet (PLT) count (183 vs. 225, p<0.001), and higher levels of ALT (23.0 vs. 16.0, p<0.001), AST (23.0 vs. 20.5, p<0.001) and total bilirubin (TB) (11.8 vs. 10.4, p=0.027). 24.9% (90/362) CHB patients were HBeAg-positive. Median level of HBV DNA was 50 IU/ml (10-4.93×10^7 IU/ml). 68.8% (249/362) CHB patients were under antiviral treatment. 13.3% (48/362) CHB patients were compensated liver cirrhosis. Moreover, the characteristics of participant at 1, 2 and 3 months after full-course vaccination are shown in Supplementary Table 1.

For analyzing, CHB patients were divided into four groups: inactive HBsAg carriers (IHCs) (n = 99, patients with normal ALT, negative HBeAg and HBV DNA＜2000 IU/ml), HBeAg-positive (+) CHB patients (n = 73, patients under antiviral treatment, with elevated or normal ALT, positive HBeAg), HBeAg-negative (-) CHB patients (n = 142, patients under antiviral treatment, with elevated or normal ALT, negative HBeAg), and CHB patients with cirrhosis (n = 48).

2. Safety of COVID-19 vaccination in CHB patients

The overall incidence of AEs within 7 days was similar between CHB patients and

healthy controls (14.1% vs. 11.5%, p=0.526) (Table 2). The most common local and systemic AEs of CHB patients were pain (5.8%, 21/362) and fatigue (4.7%, 17/362), respectively, which was similar with controls. All the AEs were mild (grade 1 and 2) and self-limiting, and no serious adverse events (SAEs) (grade 3 and 4) such as severe thromboembolism and myocarditis occurred. After prolonging the observation duration to 30 days, only three new cases with mild AEs were reported in CHB patients, including injection site pain (1 patient), fatigue (1 patient) and fever (1 patient). In healthy controls, no more AE was observed.

3. Antibody responses to COVID-19 vaccination

Three antibodies, including anti-spike IgG, anti-RBD IgG, and RBD-ACE2 blocking antibody, were determined in 362 CHB patients (99 inactive HBsAg carriers (IHCs), 73 HBeAg+ CHB patients, 142 HBeAg- CHB patients and 48 CHB patients with cirrhosis) and 87 healthy controls at 1, 2 and 3 months after full-course vaccination. At 1, 2 and 3 months, seropositivity rates of three antibodies were similar between CHB patients and healthy controls (Table 3). Also, at 1, 2 and 3 months, seropositivity rates of three antibodies were similar between IHCs, HBeAg+ CHB patients, HBeAg- CHB patients, cirrhotic patients and healthy controls (Supplementary Table 2).

But for antibody titers, at 1 month, all three antibody titers were lower in CHB patients than in healthy controls (Figure 1, data are shown in Supplementary Table 3). For anti-spike IgG, titers were significantly lower in all CHB patients (130.1 vs. 200.0, p=0.008), IHCs (121.0 vs. 200.0, p=0.031) and HBeAg- CHB patients (122.5 vs. 200.0, p=0.027) than in controls. For anti-RBD IgG, titers were significantly lower in all CHB

patients (243.7 vs. 357.7, p=0.015) and IHCs (211.2 vs. 357.7, p=0.014) than in controls. For RBD-ACE2 blocking antibody, titers were also lower in all CHB patients (6.6 vs. 8.0, p=0.549) than in controls.

At 2 month, no difference was found in titers of all three antibodies between CHB patients and healthy controls (Figure 1, data are shown in Supplementary Table 3). Interestingly, at 3 month, titers of anti-spike IgG, anti-RBD IgG and RBD-ACE2 blocking antibody were all higher in CHB patients (especially in HBeAg+ CHB patients (161.6 vs. 85.9, p=0.024, 275.4 vs. 132.5, p=0.032, 4.7 vs. 3.2, p=0.089, respectively)) than in controls. As is shown in Supplementary Figure 2, compared to controls, CHB patients, especially HBeAg+ CHB patients, had a slower decline in all three antibody titers. Also, statistical results showed that titers of anti-spike IgG (200.0 vs. 133.5 vs. 85.9 at 1, 2 and 3 months, respectively, $p<0.001$), anti-RBD IgG (357.7 vs. 252.0 vs. 132.5, $p<0.001$), and RBD-ACE2 blocking antibody (8.0 vs. 4.2 vs. 3.2, $p<0.001$) declined significantly over time in controls, but not in HBeAg+ CHB patients (all $p\geq 0.05$).

Moreover, at 1, 2 and 3 months, no difference was found in all three antibody titers between IHCs, HBeAg+ CHB patients, HBeAg- CHB patients and cirrhotic patients (Supplementary Figure 3, data are shown in Supplementary Table 4). Besides, titers of three antibodies were positively correlated with each other (Supplementary Figure 4).

4. RBD-specific B cell responses to COVID-19 vaccination

To furtherly investigate humoral immune response to vaccine, the frequency and phenotype of RBD-specific B cells were also detected at 1, 2 and 3 months after full-

course vaccination. At 1, 2 and 3 months, no difference was found in the frequencies of RBD-specific B cells, RBD-specific memory B cells (MBCs), RBD^+ activated MBCs, RBD^+ resting MBCs and RBD^+ intermediate MBCs between all CHB patients and healthy controls (Figure 2A, data are shown in Supplementary Table 5). At 1 and 2 months, compared to controls, all CHB patients (including HBeAg+ CHB patients) had lower frequency of RBD^+ atypical memory B cells (21.6% vs. 17.0%, p=0.088, 21.1% vs. 17.2%, p=0.047, respectively). At 3 month, frequency of RBD^+ atypical memory B cells was higher in HBeAg+ CHB patients than in controls (23.6% vs. 16.7%, p=0.035) (Figure 2B). Besides, frequency of RBD^+ atypical memory B cells declined significantly over time in controls (21.6% vs. 21.1% vs. 16.7% at 1, 2 and 3 months, respectively, p=0.007), whereas the frequency of RBD^+ atypical memory B cells tended to increase in HBeAg+ CHB patients (16.4% vs. 17.8% vs. 23.6% at 1, 2 and 3 months, respectively, p=0.113) (Supplementary Figure 5).

5. Factors associated with antibody response to COVID-19 vaccination

Furthermore, to obtain factors that affected titers of anti-RBD IgG, demographic, clinical and immunological characteristics were analyzed by univariate and multivariate ordinal logistic regression. Days after full-course vaccination was negatively correlated with antibody titers (OR=0.978, $p<0.001$), and frequency of RBD-specific B cells was positively correlated with antibody titers (OR=1.067, p=0.004), while liver cirrhosis, antiviral treatment, levels of HBV DNA, ALT and AST and TB were not significantly correlated with titers of anti-RBD IgG ($p \geqslant 0.05$) (Supplementary Table 6). Factors that associated with titers of anti-spike IgG and

blocking antibody are shown in Supplementary Table 7 and 8.

Discussion

This study demonstrated that COVID-19 vaccines were well tolerated in IHCs, HBeAg+ CHB patients, HBeAg- CHB patients and CHB patients with compensated cirrhosis. Seropositivity rates of anti-spike IgG, anti-RBD IgG, and RBD-ACE2 blocking antibody were similar between CHB patients and healthy controls at 1, 2 and 3 months after full-course vaccination. Titers of three antibodies were lower in CHB patients than in healthy controls at 1 month, but were higher in CHB patients (especially in HBeAg+ CHB patients) than in controls at 3 month. And compared to healthy controls, HBeAg+ CHB patients appeared to have a slower decline in antibody titers. No difference was found in the titers of all three antibodies between IHCs, HBeAg+ CHB patients, HBeAg- CHB patients and cirrhotic patients.

As for AE, the study showed that CHB patients and healthy controls had similar overall incidence within 7 days and 30 days after vaccination. Importantly, all AEs in both groups were mild and self-limiting. The results in CHB patients were similar with previous studies on patients with CLD[8,9] and liver cirrhosis[9].

A comprehensive analysis on the responses of various antibodies can better evaluate humoral immune response to COVID-19 vaccine, which involved anti-spike IgG, anti-RBD IgG, and RBD-ACE2 blocking antibody. At 1 month after full-course vaccination, titers of all three antibodies were lower in CHB patients than in controls. It was partly in accord with a recent study of CLD patients, which reported 24% of CLD patients

developed poor antibody response after SARS-CoV-2 vaccination[9]. This result indicated that compared with healthy controls, humoral immune response to COVID-19 vaccination in CHB patients might be weakened at 1 month. But to what extent will the reduction in CHB patients' antibody titers influence the protective efficacy of vaccine needs field epidemiological investigation. At 1 month, frequency of RBD-specific B cells was lower in IHCs than in controls, so were the titers of anti-RBD IgG. And frequency of RBD-specific B cells was positively correlated with titers of anti-RBD IgG. It indicated that reduction in frequency of RBD-specific B cells might partly be correlated with lower titers of anti-RBD IgG at 1 month. At 3 month, titers of all three antibodies were higher in HBeAg+ CHB patients than in healthy controls. Meanwhile, frequency of RBD^+ atypical MBCs was also higher in HBeAg+ CHB patients than in controls at 3 month. Moreover, though not statistically significant, frequency of RBD^+ atypical MBCs was positively correlated with titers of anti-RBD IgG. Atypical MBCs, a subset of MBCs, are usually at high frequencies in chronic diseases[15-17]. A previous study has shown that atypical memory B cell is a short-lived activated cell that may represent a precursor plasma cell (PC) population[18]. Therefore, higher frequency of RBD^+ atypical MBCs in HBeAg+ CHB patients might result in higher frequency of PCs, which might in turn partly led to higher antibody titers. Besides, compared to healthy controls, HBeAg+ CHB patients appeared to have a slower decline in antibody titers, and follow-up is still going on to explore this interesting phenomenon.

Levels of HBV DNA[19,20] and ALT[19] may influence the immune state of patients

infected with HBV. In this study, levels of HBV DNA and ALT were not correlated with antibody titers of anti-RBD titers. Thuluvath et al.[9] found cirrhosis was not associated with a poor antibody response after COVID-19 vaccination using multivariate analysis. Similarly, in this study, cirrhotic CHB patients and non-cirrhotic CHB patients had similar antibody titers.

Memory B cells is the pivot element for a quick antibody response in case of re-infection[21]. Previous studies have shown that SARS-CoV-2-specific memory B cells can be retained in an infected individual for at least 5-6 months[22-24], or even as long as 1 year[25]. In this study, about three months after full-course vaccination, SARS-CoV-2-specific memory B cells can still be detected in CHB patients and healthy individuals, and the frequency of RBD-specific memory B cells was similar between CHB patients and healthy controls.

The strengths of this study are as follows: Firstly, this is the first study, with healthy people as controls, focusing on the safety and antibody response of COVID-19 vaccination in CHB patients, which provides evidence for clinical practice. Secondly, responses of three antibodies were analyzed to comprehensively assess humoral response to vaccine. Thirdly, B cell responses were also determined to explore the mechanism of antibody response, and frequencies of RBD-specific B cells and RBD^+ atypical MBCs were found to be probably correlated with antibody titers. However, this study does have limitations. First, data in this study were only up to 105 days. Second, antibody response is only part of the immunogenicity of COVID-19 vaccine, so there is a need to explore T cell response. And we are exploring T cell response in our on-

going study. Third, though several criteria were used to exclude participants with the history of SARS-CoV-2 infection, there was possibility that a very few asymptomatic/mild cases might be enrolled because we did not have the baseline of antibodies to SARS-CoV-2.

In conclusion, this study comprehensively analyzed the safety, antibody response and B cell response of inactivated COVID-19 vaccination in CHB patients. COVID-19 vaccines were well tolerated in CHB patients with/without cirrhosis. Seropositivity rates of three antibodies were similar between CHB patients and healthy controls at 1, 2 and 3 months after full-course vaccination, but CHB patients had lower antibody titers at 1 month. Compared to healthy controls, HBeAg+ CHB patients had higher antibody titers at 3 month and a slower decline in antibody titers. So, inactivated COVID-19 vaccines were well tolerated, and induced effective antibody response against SARS-CoV-2 in CHB patients.

Reference

1. Wang J, Lu Z, Jin M, et al. Clinical characteristics and risk factors of COVID-19 patients with chronic hepatitis B: a multi-center retrospective cohort study. *Front Med.* 2021.
2. Kovalic AJ, Satapathy SK, Thuluvath PJ. Prevalence of chronic liver disease in patients with COVID-19 and their clinical outcomes: a systematic review and meta-analysis. *Hepatol Int.* 2020;14:612-620.
3. Wu J, Yu J, Shi X, et al. Epidemiological and clinical characteristics of 70 cases of coronavirus disease and concomitant hepatitis B virus infection: A multicentre descriptive study. *J Viral Hepat.* 2021;28:80-88.
4. Razavi-Shearer D, Gamkrelidze I, Nguyen MH, et al. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *The Lancet Gastroenterology & Hepatology.* 2018;3:383-403.
5. Sadoff J, Gray G, Vandebosch A, et al. Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. *N Engl J Med.* 2021;384:2187-2201.
6. John BV, Deng Y, Scheinberg A, et al. Association of BNT162b2 mRNA and mRNA-1273 Vaccines With COVID-19 Infection and Hospitalization Among Patients With Cirrhosis. *JAMA Internal Medicine.* 2021;181.
7. Thompson MG, Burgess JL, Naleway AL, et al. Prevention and Attenuation of Covid-19 with the BNT162b2 and mRNA-1273 Vaccines. *N Engl J Med.* 2021;385:320-329.
8. Wang J, Hou Z, Liu J, et al. Safety and immunogenicity of COVID-19

vaccination in patients with non-alcoholic fatty liver disease (CHESS2101): A multicenter study. *J Hepatol*. 2021;75:439-441.

9. Thuluvath PJ, Robarts P, Chauhan M. Analysis of antibody responses after COVID-19 vaccination in liver transplant recipients and those with chronic liver diseases. *J Hepatol.* 2021.
10. Ruether DF, Schaub GM, Duengelhoef PM, et al. SARS-CoV2-specific Humoral and T-cell Immune Response After Second Vaccination in Liver Cirrhosis and Transplant Patients. *Clin Gastroenterol Hepatol.* 2021.
11. Maini MK, Gehring AJ. The role of innate immunity in the immunopathology and treatment of HBV infection. *J Hepatol.* 2016;64:S60-S70.
12. Maini MK, Burton AR. Restoring, releasing or replacing adaptive immunity in chronic hepatitis B. *Nat Rev Gastroenterol Hepatol.* 2019;16:662-675.
13. Gehring AJ, Protzer U. Targeting Innate and Adaptive Immune Responses to Cure Chronic HBV Infection. *Gastroenterology.* 2019;156:325-337.
14. Xu XY, Ding HG, Li WG, et al. Chinese guidelines on the management of liver cirrhosis (abbreviated version). *World J Gastroenterol.* 2020;26:7088-7103.
15. Charles ED, Brunetti C, Marukian S, et al. Clonal B cells in patients with hepatitis C virus-associated mixed cryoglobulinemia contain an expanded anergic CD21low B-cell subset. *Blood.* 2011;117:5425-5437.
16. Moir S, Ho J, Malaspina A, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J Exp Med.* 2008;205:1797-1805.

17. Weiss GE, Crompton PD, Li S, et al. Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. *J Immunol.* 2009;183:2176-2182.

18. Jenks SA, Cashman KS, Zumaquero E, et al. Distinct Effector B Cells Induced by Unregulated Toll-like Receptor 7 Contribute to Pathogenic Responses in Systemic Lupus Erythematosus. *Immunity.* 2018;49:725-739 e726.

19. Gao P, Luo Y, Chen L, Yang Z, He Q, Li J. The effect of hepatitis B virus on T lymphocyte and its subsets in chronic hepatitis B patients in different ALT stages: A new concept ALT in HBV infection. *Int Immunopharmacol.* 2021;101:108182.

20. Tjwa ET, van Oord GW, Hegmans JP, Janssen HL, Woltman AM. Viral load reduction improves activation and function of natural killer cells in patients with chronic hepatitis B. *J Hepatol.* 2011;54:209-218.

21. Kurosaki T, Kometani K, Ise W. Memory B cells. *Nat Rev Immunol.* 2015;15:149-159.

22. Gaebler C, Wang Z, Lorenzi JCC, et al. Evolution of antibody immunity to SARS-CoV-2. *Nature.* 2021;591:639-644.

23. Sokal A, Chappert P, Barba-Spaeth G, et al. Maturation and persistence of the anti-SARS-CoV-2 memory B cell response. *Cell.* 2021;184:1201-1213 e1214.

24. Sakharkar M, Rappazzo CG, Wieland-Alter WF, et al. Prolonged evolution of the human B cell response to SARS-CoV-2 infection. *Sci Immunol.* 2021;6.

25. Wang Z, Muecksch F, Schaefer-Babajew D, et al. Naturally enhanced

neutralizing breadth against SARS-CoV-2 one year after infection. *Nature.* 2021;595:426-431.

Table 1 Characteristics of participants

Variables	Healthy controls (n=87)	Patients (n=362)	*P* value
Age[#] (years)	44.0 (25-75)	45.0 (19-78)	0.143
18-40, n (%)	37 (42.5%)	142 (39.2%)	0.572
≥40, n (%)	50 (57.5%)	220 (60.8%)	
Gender (male, n (%))	44 (50.6%)	223 (61.6%)	0.060
BMI[#] (kg/m^2)	23.6 (17.0-36.4)	23.4 (16.2-32.8)	0.752
＜24, n (%)	50 (57.5%)	217 (59.9%)	
24-28, n (%)	32 (36.8%)	122 (33.7%)	0.857
≥28, n (%)	5 (5.7%)	23 (6.4%)	
WBC[#] (10^9/L)	5.70 (3.52-11.32)	5.37 (2.59-11.65)	**0.039**
PLT[#] (10^9/L)	225 (111-338)	183 (22-320)	**0.000**
ALT[#] (U/L)	16.0 (7-45)	23.0 (6-802)	**0.000**
AST[#] (U/L)	20.5 (11-30)	23.0 (12-352)	**0.000**
TB[#] (umol/L)	10.4 (4.5-23.8)	11.8 (4.5-53.0)	**0.027**
HBeAg (positive, n (%))	/	90 (24.9%)	/
HBV DNA[#] (IU/ml)	/	50 (10 - 4.93×10^7)	/
Antiviral treatment, n (%)	/	249 (68.8%)	/
Liver cirrhosis, n (%)	/	48 (13.3%)	/

[#]Presented as median (Range). Notes: When HBV DNA level is lower than detection limit (20 IU/ml), 10 IU/ml is assigned. Patients with liver cirrhosis were in compensatory stage. Chi-Square statistic test was used for categorical variables, and Mann-Whitney U test was used for continuous variables. $p < 0.05$ was

considered statistically significant.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; PLT, platelet; TB, total bilirubin; WBC, white blood cell

Table 2 Adverse events of COVID-19 vaccination in participants

	Healthy controls (n=87)	Patients (n=362)	*P* value
Overall adverse events within 7 days	10 (11.5%)	51 (14.1%)	0.526
Overall adverse events within 30 days	10 (11.5%)	54 (14.9%)	0.412
Local adverse events			
Pain	9 (10.3%)	21 (5.8%)	0.128
Swelling	1 (1.1%)	3 (0.8%)	0.579
Redness	1 (1.1%)	/	0.194
Systemic adverse events			
Fatigue	2 (2.3%)	17 (4.7%)	0.483
Dizziness	/	7 (1.9%)	0.409
Diarrhea	/	2 (0.6%)	1.000
Laryngeal pain	/	2 (0.6%)	1.000
Cough	1 (1.1%)	1 (0.3%)	0.350
Chest distress	/	1 (0.3%)	1.000
Chest pain	/	1 (0.3%)	1.000
Chill	/	1 (0.3%)	1.000
Elevated blood pressure	/	1 (0.3%)	1.000
Fever	/	1 (0.3%)	1.000
Inappetence	/	1 (0.3%)	1.000
Muscle pain	/	1 (0.3%)	1.000
Nausea	/	1 (0.3%)	1.000

Palpitation	/	1 (0.3%)	1.000
Pruritus	/	1 (0.3%)	1.000
Grade 3 and 4 adverse events	/	/	1.000

Data are presented as n (%). Chi-square test and Fisher's exact test were used to compare statistical difference between groups. $p < 0.05$ was considered statistically significant.

Table 3 Seropositivity rates of SARS-CoV-2 antibodies after full-course vaccination

Antibody	Month after full-course vaccination	Healthy controls (n=87)	CHB patients (n=362)	*P* value
Spike-specific IgG	1 Month	100.0% (31/31)	97.8% (175/179)	1.000
	2 Month	100.0% (24/24)	95.7% (112/117)	0.588
	3 Month	93.8% (30/32)	95.5% (63/66)	0.660
RBD-specific IgG	1 Month	100.0% (31/31)	98.3% (176/179)	1.000
	2 Month	95.8% (23/24)	97.4% (114/117)	0.530
	3 Month	96.9% (31/32)	98.5% (65/66)	0.549
Blocking antibody	1 Month	77.4% (24/31)	72.6% (130/179)	0.577
	2 Month	45.8% (11/24)	45.3% (53/117)	0.962
	3 Month	28.1% (9/32)	43.9% (29/66)	0.132

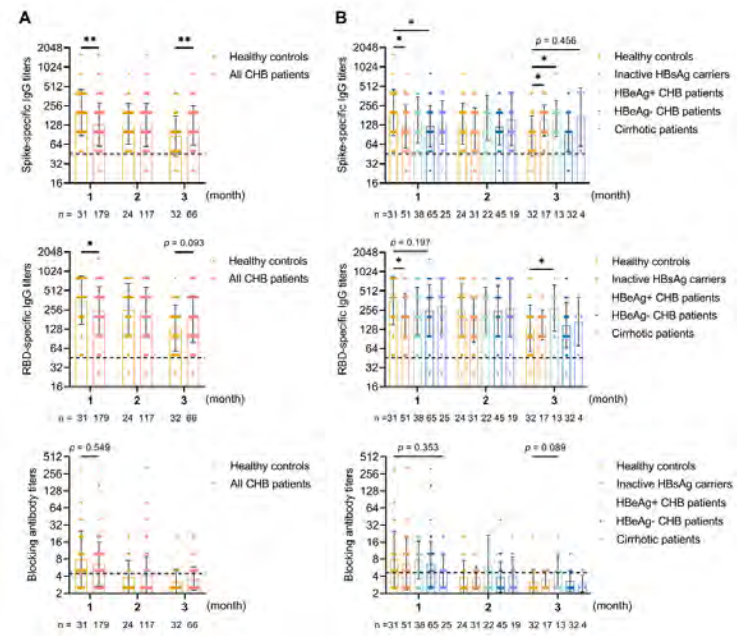
Chi-square test and Fisher's exact test were used to compare statistical difference of seropositivity rates of antibodies at 1, 2 and 3 months after full-course vaccination between healthy controls and CHB patients. RBD, receptor binding domain. $p < 0.05$ was considered statistically significant.

Figure legends

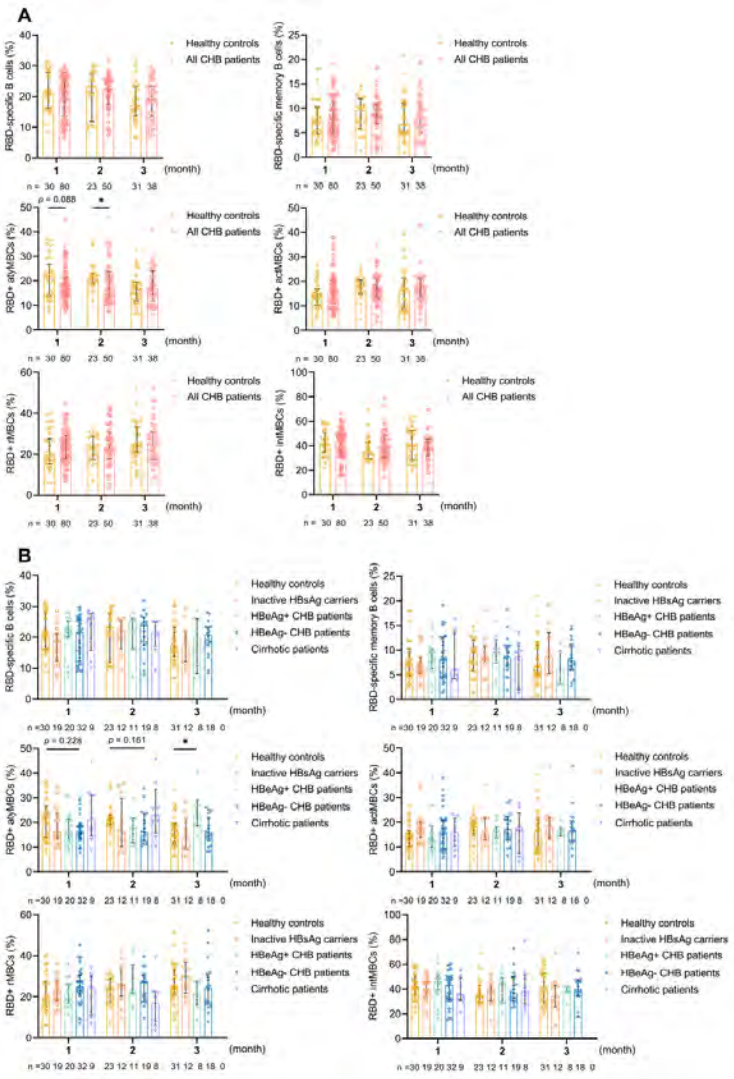
Figure 1 Antibody response after full-course vaccination in CHB patients and healthy controls. **(A)** Titers of spike-specific IgG, RBD-specific IgG, blocking antibody in healthy controls (n = 87) and all CHB patients (n = 362) at 1, 2 and 3 months after full-course vaccination. **(B)** Titers of spike-specific IgG, RBD-specific IgG, blocking antibody in healthy controls (n = 87), inactive HBsAg carriers (n = 99), HBeAg+ CHB patients (n = 73), HBeAg- CHB patients (n = 142) and cirrhotic patients (n = 48) at 1, 2 and 3 months after full-course vaccination. Mann-Whitney U test was used for two-group comparison (healthy controls and all CHB patients). Dunn's multiple comparisons test was used for comparisons between healthy controls and CHB subgroups, and the results were corrected. Top of all bars represents GMTs and error bars represent geometric SD. Horizontal dotted lines represent limit of detection. When the antibody titer of participant was lower than the detection limit, half value of the detection limit was assigned. The p-values represented in this figure are all adjusted p-values. *p<0.05, **p<0.01. GMTs, geometric mean titers; RBD, receptor binding domain; SD, standard deviation.

Figure 2 RBD-specific B cell responses at 1, 2 and 3 months after full-course vaccination in CHB patients and healthy controls. **(A)** Frequency of RBD-specific cell and its subsets in healthy controls (n = 84) and all CHB patients (n = 168). According to top-to-bottom and left-to-right order, plots represent the percentage of RBD-specific B cells in B cells, percentage of RBD-specific MBCs in B cells, and percentage of four

RBD-specific MBC subsets: RBD+ atypical MBCs, RBD+ activated MBCs, RBD+ resting MBCs and RBD+ intermediate MBCs. **(B)** Frequency of RBD-specific cell and its subsets in healthy controls (n = 84), inactive HBsAg carriers (n = 43), HBeAg+ CHB patients (n = 39), HBeAg- CHB patients (n = 69) and cirrhotic patients (n = 17). Mann-Whitney U test was used for two-group comparison (healthy controls and all CHB patients). Dunn's multiple comparisons test was used for comparisons between healthy controls and CHB subgroups, and the results were corrected. Top of all bars represents median and error bars represent IQR. The p-values represented in this figure are all adjusted p-values. *$p < 0.05$. IQR, interquartile range; MBC, memory B cell; RBD, receptor binding domain.



LIV_15173_Figure 1.tif



LIV_15173_Figure 2.tif

CoronaVac

O que a ciência comprova

4.5. CoronaVac induz anticorpos em 85,2% dos pacientes com câncer, mostra estudo turco

Um estudo publicado na revista Future Oncology mostrou que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, é eficaz na proteção de pessoas com câncer, induzindo a produção de altos títulos de anticorpos em 85,2% dos pacientes analisados. O trabalho foi realizado por pesquisadores turcos da Universidade Bezmialem Vakif, Universidade Medipol, Hospital de Treinamento e Pesquisa Okmeydani, Hospital de Ancara, entre outras instituições.

Os cientistas avaliaram a soropositividade da CoronaVac em 776 pacientes com câncer, adultos com idade média de 64 anos, que deram entrada em clínicas de oncologia entre 1/3 1/7 de 2021. O grupo controle foi composto por 715 pessoas sem câncer, com idade média de 50 anos. Todos foram vacinados com duas doses, com intervalos de quatro a seis semanas.

Entre os pacientes, 85,2% produziram anticorpos contra o SARS-CoV-2, com título mediano de 363,9 UA/mL. Já no grupo controle, a taxa de soropositividade foi de 97,5% e o título mediano de anticorpos foi de 656,5 UA/mL.

A incidência de efeitos adversos após a primeira dose foi de 15,9% no grupo de pacientes e de 22,5% no grupo controle, sendo que os sintomas mais relatados foram fadiga e dor no local da injeção. Em relação à segunda dose, não houve diferença significativa nas reações adversas.

Os tipos tumorais mais comuns foram câncer de mama (32,3%), câncer de pulmão (23,6%), câncer gastrointestinal (22,4%) e câncer geniturinário (13,8%). Dos pacientes, 51,3% (398 pessoas) apresentavam doença metastática; 39,8% (309 pessoas) estavam em quimioterapia ativa; 15,1% (117 pessoas) estavam

em imunoterapia ou terapias direcionadas; e 45,1% (350 pessoas) não receberam nenhuma dessas modalidades de tratamento nos três meses anteriores.

De acordo com os pesquisadores, os fatores significativamente associados às menores taxas de soropositividade no grupo de pacientes foram idade e quimioterapia ativa. No entanto, os resultados confirmam a eficácia e a segurança da CoronaVac nessa população.

Diferenças de soropositividade entre os pacientes

Para comparar as taxas de produção de anticorpos entre os pacientes, os cientistas dividiram os participantes em quatro subgrupos: grupo de quimioterapia ativa, grupo de imunoterapia, grupo de terapias direcionadas e grupo de terapias hormonais.

As taxas de soropositividade foram de 78,6% no grupo de quimioterapia ativa, 85,7% no grupo de imunoterapia, 86% no grupo de terapias direcionadas e 87,1% no grupo de terapia hormonal. Para os pacientes que não receberam nenhum tratamento, a taxa de soropositividade foi de 91,1%. Além disso, 90,7% dos pacientes sem metástase e 79,9% dos pacientes com metástase produziram anticorpos.

Publicado em: 27/1/2022

esearch Article

Efficacy and safety profile of COVID-19 vaccine in cancer patients: a prospective, multicenter cohort study

yse Irem Yasin*,[1], Sabin Göktas Aydin[2], Bilge Sümbül[3], Lokman Koral[4], Melih imşek[1], Çağlayan Geredeli[5], Akın Öztürk[6], Perihan Perkin[7], Derya Demirtaş[8], Engin rdemoglu[9], İlhan Hacıbekiroglu[10], Emre Çakır[10], Eda Tanrıkulu[11], Ezgi Çoban[11], Melike zcelik[12], Sinemis Çelik[13], Fatih Teker[14], Asude Aksoy[15], Sedat T Fırat[16], Ömer Tekin[17], Ziya alkan[18], Orhan Türken[19], Bala B Oven[20], Faysal Dane[21], Ahmet Bilici[2], Abdurrahman ıkdogan[18], Mesut Seker[1], Hacı M Türk[1] & Mahmut Gümüş[9]

Bezmialem Vakif University, Department of Medical Oncology, Istanbul 34093, Turkey
Medipol University, Department of Medical Oncology, Istanbul 34214, Turkey
Bezmialem Vakif University, Department of Microbiology, Istanbul 34093, Turkey
Canakkale 18 March University, Department of Medical Oncology, Canakkale 17020 ,Turkey
Okmeydani Training and Research Hospital, Department of Medical Oncology, Istanbul 34384, Turkey
SureyyapasaChest Diseases And Thoracic Surgery Trainıng And Research Hospıtal, Department of Medical Oncology, Istanbul 4844, Turkey
Yildirim Beyazit University Yenimahalle Training and Research Hospital, Department of Medical Oncology, Ankara 06330, Turkey
AnkaraCity Hospital, Department of Medical Oncology, Ankara06800, Turkey
GöztepeMedeniyet University, Department of Medical Oncology, Istanbul 34000, Turkey
Sakarya University Medicine Faculty, Departmentof Medical Oncology, Sakarya 54050, Turkey
Haydarpasa Training and Research Hospital, University of Health Sciences, Istanbul 34668, Turkey
Marmara University School of Medicine, Department of Medical Oncology, Istanbul 34722, Turkey
Istanbul Oncology Hospital, Department of Medical Oncology, Istanbul 34846, Turkey
Gaziantep University, Department of Medical Oncology, Gaziantep 27470, Turkey
Fırat University Faculty of Medicine, Department of Medical Oncology, Elazıg 23119, Turkey
Erciyes University, Department of Medical Oncology, Kayseri 38039, Turkey
InönüUniversity, Department of Medical Oncology, Malatya 44280, Turkey
DicleUniversity, Department of Medical Oncology, Diyarbakır 21200, Turkey
MaltepeUniversity, Department of Medical Oncology, Istanbul 34844, Turkey
Bahcesehir University School of Medicine, Department of Medical Oncology, Istanbul 34349, Turkey
Acıbadem University, Department of MedicalOncology, Istanbul 34758, Turkey
Author for correspondence: Tel.: +90 212 453 1700; ayseiremyasin@gmail.com

im: To compare the seropositivity rate of cancer patients with non-cancer controls after inactive SARS-oV-2 vaccination (CoronaVac) and evaluate the factors affecting seropositivity. **Method:** Spike IgG ntibodies against SARS-CoV-2 were measured in blood samples of 776 cancer patients and 715 non-ancer volunteers. An IgG level ≥50 AU/ml is accepted as seropositive. **Results:** The seropositivity rate was 5.2% in the patient group and 97.5% in the control group. The seropositivity rate and antibody levels ere significantly lower in the patient group ($p < 0.001$). Age and chemotherapy were associated with ower seropositivity in cancer patients ($p < 0.001$). **Conclusion:** This study highlighted the efficacy and afety of the inactivated vaccine in cancer patients.

Clinical Trials Registration: NCT04771559 (ClinicalTrials.gov)

lain language summary: Cancer patients are at high risk for infection with SARS-CoV-2 and of developing ne associated disease, COVID-19, which therefore puts them in the priority group for vaccination. This tudy evaluated the efficacy and safety of CoronaVac, an inactivated virus vaccine, in cancer patients. The mmune response rate, defined as seropositivity, was 85.2% in the cancer patient group and 97.5% in ne control group. The levels of antibodies, which are blood markers of immune response to the vaccine, ere also significantly lower in the patient group, especially in those older than 60 years and receiving hemotherapy. These results highlight the importance of determining the effective vaccine type and dose n cancer patients to protect them from COVID-19 without disrupting their cancer treatment.

Future Medicine

First draft submitted: 1 October 2021; Accepted for publication: 9 December 2021; Published online: 27 January 2022

Keywords: cancer • chemotherapy • CoronaVac • COVID-19 • COVID-19 vaccines • immunotherapy • malignancy • SARS-CoV-2

COVID-19, which emerged in China in 2019 and spread all over the world in a short time, caused many deaths around the world [1]. In many countries, including Turkey, measures are continuing to prevent the spread of the virus, which has many negative effects on social and economic life. Since the beginning of the pandemic, many countries have carried out studies to develop a vaccine against COVID-19. Today there are more than ten different vaccines currently in use worldwide [2]. Turkey's national immunization program continues by prioritizing high-risk groups such as elderly adults and cancer patients. Approximately 70% of the population has been vaccinated with at least two doses [3].

Studies have shown that the morbidity and mortality of COVID-19 in cancer patients are higher than in non-cancer individuals [4–6]. COVID-19 progresses more severely in cancer patients due to the natural course of the cancer and the oncological treatments [7,8].

Cancer patients were also negatively affected by disruptions in cancer diagnosis and treatment during the pandemic. A European survey showed an average reduction of 29.3% in all types of oncological surgeries [9]. Riera *et al.* reviewed delays and disruptions in cancer management due to the pandemic; they reported up to 77.5% interruption in any stage of cancer treatment [10]. As a result of interruptions in oncological diagnosis and treatment processes, the increase in cancer-related deaths in England over the past year was estimated to be 20% [11].

The COVID-19 seroprevalence in cancer patients was evaluated in recent studies. Fillmore *et al.* screened the results of 22,914 cancer patients tested for COVID-19 and reported 7.8% positivity [12]. In another study, 928 cancer patients with a COVID-19 diagnosis were evaluated, and 4% were reported as asymptomatic [13]. The leading oncological societies, such as the American Society of Clinical Oncology, European Society of Medical Oncology and National Comprehensive Cancer Network (NCCN), have developed guidelines to minimize the negative effects of the COVID-19 pandemic on cancer patients. However, there is no consensus for SARS-CoV-2 testing of asymptomatic patients before initiation of immunosuppressive therapies [14]. An individual risk–benefit assessment for each patient appears to be the most reliable method yet [14].

Because there is no standard treatment for COVID-19, vaccination is considered to be the cornerstone for mitigation of the pandemic. The severe course of COVID-19 in cancer patients puts them among the priority groups for vaccination. The NCCN recommends that people with active cancer undergoing treatment, those about to be treated for cancer and those who have been treated for cancer in the past 6 months should be prioritized to receive vaccinations as soon as possible [15]. Different types of COVID-19 vaccines are currently available around the world. CoronaVac, an inactivated vaccine, is one of the most applied vaccines. Solodky *et al.* reported that the antibody level in cancer patients after COVID-19 was lower than that in healthy individuals [16]. A similar situation is expected to be seen in the post-vaccine antibody response. Although the seroconversion rate in healthy adults after two doses of inactivated vaccine was reported as 100% in the CoronaVac study, seroconversion in cancer patients was not assessed [17]. In another study evaluating the efficacy of CoronaVac, the seropositivity rate was 89.7% [18]. Furthermore, the seroconversion rate of the BNT162b2 mRNA vaccine was found to be 95% in healthy adults [19]. Currently, limited data are available showing the efficacy and safety of COVID-19 vaccines in cancer patients. Ariamanesh *et al.* recently demonstrated 86.9% seropositivity after administration of inactivated vaccine in patients with malignancy [20]. Massarweh *et al.* reported 90% seropositivity in 102 cancer patients vaccinated with the BNT162b2 mRNA vaccine [21]. However, the role of COVID-19 vaccination remains a challenging issue in cancer patients.

In this study we aimed to compare cancer patients with non-cancer controls in terms of the efficacy and safety of inactive SARS-CoV-2 (CoronaVac) vaccination. In addition, factors affecting seropositivity in cancer patients were evaluated.

This trial is registered with ClinicalTrials.gov (NCT04771559) and is closed to accrual.

Patients & methods

Study design

This study is a prospective, multicenter cohort study evaluating the efficacy and safety of the CoronaVac in cancer patients. Initially, 2154 adult patients with histologically diagnosed solid tumors who were admitted to medical

ology clinics between 1 March and 1 July 2021 were informed about the study; the control group consisted of thcare workers and volunteers accompanying the patients. From this initial group, 776 cancer patients and 715 -cancer volunteers who received a second dose of inactivated vaccine in 4–6 weeks were included in the study. cination information and the COVID-19 history of the participants were checked from the national health rd database. Patients and controls who had a documented COVID-19 infection (positive PCR test result) at time before enrollment and patients who received an mRNA vaccine were excluded. In addition, controls who e pregnant or had an immunosuppressive disease or were receiving immunosuppressive therapy for any reason e excluded from the study. The study was carried out with permission of the Turkish Ministry of Health and roved by the local ethics committee (02/28). All participants signed a written informed consent form.

essments

od samples were taken from the patients and centrifuged at 2500 rpm for 10 min. The separated serum samples e backed up in two Eppendorf tubes and stored at -80 or -20°C. All serum samples were delivered by cold chain collected in a single center. A US FDA-approved chemiluminescent microparticle immunoassay, the Abbott nitect i1000sr SARS-CoV-2 IgG II Quant assay (Abbott Laboratories, IL, USA), was used to quantify IgG bodies against the SARS-CoV-2 spike receptor-binding domain following the manufacturer's instructions [22]. s assay has 98.1% sensitivity and 99.6% specificity at least 15 days after first symptom onset or documented VID-19 infection [23]. An IgG level ≥50 AU/ml is accepted as seropositive.

atient characteristics were collected and included age, sex, BMI, smoking status, comorbidities and receipt ny other vaccination (influenza or pneumococci) within 2 years. All participants were asked about local and emic side effects of vaccination. Additionally, all clinical information about the cancer diagnosis (tumor type, ase stage and treatment status) were recorded. Treatment groups were: chemotherapy group (including taxane, in, fluorouracil, gemcitabine, anthracycline, cyclophosphamide, pemetrexed); immunotherapy group (including olumab, pembrolizumab and atezolizumab); targeted therapies group (tyrosine kinase inhibitors, anti-VEGF nts, trastuzumab, pertuzumab, CDK4/6 inhibitors); and hormonal therapies group (tamoxifen, aromatase bitors, LHRH analogs). We evaluated each treatment group for seropositivity. Additionally, we created another up for those receiving active targeted or immunotherapies and compared the seropositivity rates of this group n those of the active chemotherapy group.

tistical analysis

criptive statistics are shown as mean ± standard deviation for variables with normal distribution, median nimum to maximum) for non-normal distributions, and the number of cases and percentage (%) for nominal ables. The Mann–Whitney U-test was used for comparison of the groups. Pearson's χ-square or Fisher's exact s were performed for nominal variables. Multivariate analysis was applied with a logistic regression test. A lue < 0.05 was considered to be statistically significant. SPSS for Windows (v. 22; IBM Corp., NY, USA) was l to analyze the data.

ults

study group consisted of 776 cancer patients and 715 non-cancer controls. The median age in the patient up was 64 years (range: 20–88), and the median age in the control group was 50 years (range: 21–94). The acteristics of the study participants are shown in Table 1.

he seropositivity rate was 85.2% and the median antibody titer was 363.9 AU/ml in the patient group. The positivity rate was 97.5% and the median antibody titer was 656.5 AU/ml in the control group. When the groups were compared, the seropositivity rate and antibody levels were significantly lower in the patient group n in the non-cancer controls ($p < 0.001$). Additionally, administration of influenza and pneumococcal vaccine alence was higher in the patient group ($p < 0.001$). Vaccine features and antibody levels are shown in Table 2. While the incidence of side effects after the first dose of vaccine was 15.9% in the patient group, this rate was 6% in the control group. The rate of side effects reported after the first dose was significantly higher in the trols than the patients ($p = 0.001$). While the most common side effect in the control group was local pain %), the most common side effect in the patient group was fatigue (6.4%). When the prevalence of side effects the second dose was compared, there was no significant difference between the two groups (Table 3).

he most common tumor types were breast cancer (32.3%), lung cancer (23.6%), gastrointestinal cancer (22.4%) genitourinary cancer (13.8 %). Of the patients, 51.3% (n = 398) had metastatic disease; 39.8% (n = 309) were

Table 1. Characteristics of study participants.

Characteristic	Patient group (n = 776)		Control group (n = 715)		p-value
	n	(%)	n	(%)	
Age, median (range)	64 (20–88)		50 (21–94)		<0.001†
Age (years)					<0.001†
<60	291	37.5	614	85.9	
≥60	485	62.5	101	14.1	
Sex					0.958
Female	433	55.8	398	55.7	
Male	343	44.2	317	44.3	
BMI, median (range)	27.1 (16–48)		26.1 (18–40)		0.943
BMI					0.943
<25 kg/m^2	187	30.7	118	30.5	
≥25 kg/m^2	422	69.3	269	69.5	
Smoking					<0.001†
No	436	59.3	428	72.2	
Ex-smoker	165	22.4	16	2.7	
Yes	135	18.3	149	25.1	
Diabetes mellitus					<0.001†
No	635	81.8	666	93.1	
Yes	141	18.2	49	6.9	
Hypertension					<0.001†
No	513	66.1	616	86.2	
Yes	263	33.9	99	13.8	
Coronary disease					<0.001†
No	710	91.5	698	97.6	
Yes	66	8.5	17	2.4	
Chronic renal failure					<0.001†
No	759	97.8	714	99.9	
Yes	17	2.2	1	0.1	
Chronic liver disease					0.081
No	761	98.1	709	99.2	
Yes	15	1.9	6	0.8	
Rheumatological disease					0.816
No	766	98.7	707	98.9	
Yes	10	1.3	8	1.1	
Psychiatric disease					0.004†
No	762	98.2	713	99.7	
Yes	14	1.8	2	0.3	
Respiratory disease					0.002†
No	741	95.5	703	98.3	
Yes	35	4.5	12	1.7	
Other					0.152
No	731	94.2	686	95.9	
Yes	45	5.8	29	4.1	

Statistically significant results.

Table 2. Vaccine features and antibody levels of the study population.

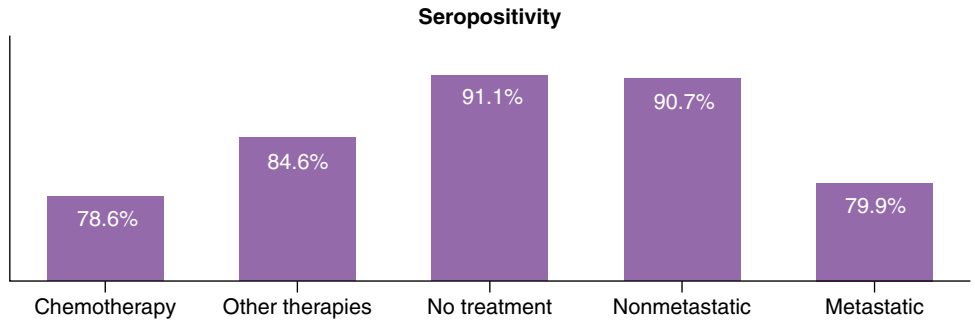
	Patient group (n = 776)		Control group (n = 715)		p-value
	n	(%)	n	(%)	
Antibody level, median (range)	363.9 AU/ml (0–40,000)		656.5 AU/ml (0.2–10,615.3)		<0.001†
Seropositivity					<0.001†
Positive (≥50)	661	85.2	697	97.5	
Negative (<50)	115	14.8	18	2.5	
Other vaccines					<0.001†
Yes	217	28.0	117	16.4	
No	559	72.0	598	83.6	
Type of vaccine					0.236
Influenza	71	32.7	48	41.0	
Pneumococcal	59	27.2	24	20.5	
Influenza + pneumococcal	87	40.1	45	38.5	

Statistically significant results.

Table 3. Side effects after the first and the second doses of the vaccine.

haracteristics	Patient group (n = 776)				Control group (n = 715)				p-value
	Total (%)	Gr 1 (%)	Gr 2 (%)	Gr 3–4 (%)	Total (%)	Gr 1 (%)	Gr 2 (%)	Gr 3–4 (%)	
irst dose	15.9				22.5				0.001†
ocal pain	5.7	5.3	0.4	–	9.7	8.3	1.1	0.3	0.005†
rythema	0.5	0.4	0.1	–	2.1	1.8	0.3	–	0.009†
ever	2.1	1.2	0.8	0.1	1.8	1.7	–	0.1	0.852
atigue	6.4	5.0	1.3	0.1	8.4	6.7	1.4	0.3	0.165
leadache	4.6	3.6	0.9	0.1	7.8	6.2	1.1	0.6	0.013†
Myalgia	4.5	3.2	0.9	0.4	6.7	4.4	2.2	0.1	0.071
lausea	1.8	1.7	–	0.1	1.4	1.4	–	–	0.681
)iarrhea	0.8	0.4	0.4	–	1.0	0.8	0.2	–	0.783
)ther	0.9	0.9	–	–	2.6	2.6	–	–	0.269
econd dose	15.2				16.8				0.436
ocal pain	5.0	4.6	0.4	–	7.7	6.2	1.3	0.3	0.042†
ever	1.3	1.0	0.3	–	2.1	2.0	0.1	–	0.234
atigue	6.7	4.9	1.5	0.3	6.4	5.1	1.0	0.3	0.917
leadache	4.5	3.7	0.8	–	4.8	3.5	0.7	0.6	0.902
Myalgia	4.9	3.4	1.0	0.5	5.9	4.3	1.0	0.6	0.422
lausea	1.2	0.8	0.4		0.7	0.7	–	–	0.427
)iarrhea	0.9	0.7	0.1	0.1	0.3	0.3	–	–	0.182
)ther	1.1	1.1	–	–	1.3	1.3	–	–	0.647

Statistically significant results.
ir: Grade.



gure 1. Seropositivity rates of cancer patients after SARS-CoV-2 vaccination according to the treatment status and age of the disease.

n active chemotherapy; 15.1% (n = 117) were on immunotherapy or targeted therapies; and 45.1% (n = 350) ad not received any of these treatment modalities within the previous 3 months. The seropositivity rates were 8.6% in the active chemotherapy group, 85.7% in the immunotherapy group, 86.0% in the targeted therapies roup and 87.1% in the hormone therapy group. For the patients not receiving any active treatment including nemotherapy, immunotherapy or targeted therapies, the seropositivity rate was 91.1% (Table 4). Additionally, 0.7% of the nonmetastatic patients and 79.9% of the metastatic patients were seropositive (Figure 1).

In univariate analysis of the patient group, chemotherapy, metastatic disease, age and male gender were negatively orrelated with seropositivity ($p < 0.001$). The seropositivity rate in the active chemotherapy group was significantly wer than in the group of patients not receiving active chemotherapy ($p < 0.001$). Tumor type, BMI, smoking nd comorbidities were not associated with seropositivity (Table 4). In univariate analysis of the control group, ge was found to be the only factor negatively correlated with seropositivity ($p < 0.001$; Table 4). When the ultivariate analysis was performed, age and chemotherapy were defined as the factors significantly associated with wer seropositivity in cancer patients ($p < 0.001$ and $p = 0.038$, respectively ; Tables 5 & 6).

Table 4. The factors affecting seropositivity in the study population.

Factors affecting seropositivity in the patient group (univariate analysis)			
haracteristics	**n (%)**	***Seropositivity (%)***	**p-value**
ge (years)			<0.001†
<60	291 (37.5)	93.5	
≥60	485 (62.5)	80.2	
ender			0.015†
Female	433 (55.8)	88.0	
Male	343 (44.2)	81.6	
MI			0.435
<25 kg/m^2	187 (30.7)	88.8	
≥25 kg/m^2	422 (69.3)	86.3	
moking			0.577
No	436 (59.2)	85.3	
Ex-smoker	165 (22.4)	87.3	
Yes	112 (17.8)	83.0	
umor type			0.335
Breast	251 (32.3)	88.0	
Gastrointestinal	174 (22.4)	86.2	
Genitourinary	107 (13.8)	84.1	
Lung	183 (23.6)	80.9	
Other	61 (7.9)	85.2	
reatment type (active)			<0.001†
No treatment	350 (45.1)	91.1	
Chemotherapy	309 (39.8)	78.6	
Targeted or IO	117 (15.1)	84.6	
hemotherapy			<0.001†
Never	152 (19.6)	87.5	
Not in the last 3 months	315 (40.6)	90.5	
Active	309 (39.8)	78.6	
mmunotherapy (IO)			0.920
Yes	42 (5.4)	85.7	
No	734 (94.6)	85.1	
argeted therapies			0.811
Yes	178 (22.9)	86.0	
No	598 (77.1)	84.9	
lormone therapy			0.426
Yes	209 (26.9)	87.1	
No	567 (73.1)	84.5	
omorbidities			0.225
No	373 (48.1)	86.9	
Yes	403 (51.9)	83.6	
tage			<0.001†
Nonmetastatic	378 (48.7)	90.7	
Metastatic	398 (51.3)	79.9	
Factors affecting seropositivity in the control group (univariate analysis)			
haracteristics	**n (%)**	***Seropositivity (%)***	**p-value**
ge (years)			<0.001†
<60	614 (85.9)	98.4	
≥60	101 (14.1)	92.1	
ender			0.347
Female	398 (55.7)	98.0	
Male	317 (44.3)	96.8	
MI			0.435
<25 kg/m^2	118 (30.5)	97.5	
≥25 kg/m^2	269 (69.5)	97.0	
moking			0.711
No	428 (72.2)	97.2	
Ex-smoker	16 (2.7)	93.8	
Yes	149 (25.1)	97.3	
omorbidities			0.399
No	544 (76.1)	97.8	
Yes	171 (23.9)	96.5	

Statistically significant results.
O: Immunotherapy.

able 5. The factors affecting seropositivity in the study population (multivariate analysis).

haracteristics	SE	RR	95% CI	p-value
oncancer vs cancer	0.286	3.519	2.009–6.162	<0.001†
ge (<60 vs ≥60)	0.246	3.545	2.190–5.737	<0.001†
ender (female vs male)	0.194	1.271	0.868–1.859	0.218
omorbidities (yes vs no)	0.195	1.129	0.771–1.655	0.533

tatistically significant results.
: Relative risk; SE: Standard error.

able 6. The factors affecting seropositivity in the patient group (multivariate analysis).

haracteristics	SE	RR	95% CI	p-value
ge (<60 vs ≥60)	0.276	3.016	1.758–5.176	<0.001†
ender (female vs male)	0.221	1.154	0.701–1.667	0.724
hemotherapy (yes vs no)	0.358	1.396	0.692–2.818	0.038†
rgeted therapy or IO (yes vs no)	0.300	0.709	0.393–1.277	0.351
omorbidities (yes vs no)	0.213	1.116	0.736–1.692	0.606
age (metastatic vs nonmetastatic)	0.304	1.458	0.804–2.645	0.214

tatistically significant results.
: Immunotherapy; RR: Relative risk; SE: Standard error.

iscussion

his study showed 85.2% seropositivity in cancer patients, whereas this rate was 97.5% in non-cancer con-ols. Additionally, IgG antibody titers in cancer patients were significantly lower than in the controls. The factors gnificantly associated with low seropositivity rates in the patient group were age and active chemotherapy. When e side effects in both groups were compared, the control group reported significantly more side effects after the st dose. Nevertheless, there was no significant difference between the groups in side effects after the second dose. ur findings confirmed the efficacy and safety of CoronaVac in cancer patients.

The COVID-19 pandemic negatively affected cancer patients. In addition to the severe course of COVID-19 cancer patients, covidophobia, delays in cancer diagnosis and disruptions to oncological treatments increased e mortality of cancer patients during the pandemic [4–11]. NCCN and other oncological societies recommended at all cancer patients, especially those receiving active treatment, should be vaccinated as a priority [15]. The gh seropositivity rate of cancer patients in our study also supports these recommendations, even though the ropositivity rate was relatively lower than in non-cancer adults.

The low seropositivity rate in cancer patients compared with the non-cancer controls found in this study was pected, as immunosuppression negatively affects the immune response. Similar to our results, Ariamanesh *et al.* und that older age, chemotherapy and hematological malignancies were related to lower seropositivity rates er administration of inactivated vaccine [20]. Massarweh *et al.* reported that chemotherapy plus immunotherapy eatment was associated with lower IgG titers in cancer patients vaccinated with the BNT162b2 mRNA vaccine [21]. rthermore, studies evaluating the response to pneumococcal and influenza vaccines in patients with malignancy owed a decreased response in patients with hematological malignancies [24]. In another study, influenza vaccine sponse was low in breast cancer patients receiving active chemotherapy [25]. Our findings also highlight the gative effect of active treatment on immune response.

Although a clear relationship has not yet been established between antibody levels and prevention of the disease, e main target of the vaccines is to trigger the formation of neutralizing antibodies against the SARS-CoV-2 ike protein [26]. Harvey *et al.* reported an approximately tenfold increase in positive nucleic acid amplification st results among patients with positive antibody tests compared with those who had negative antibody tests, ggesting a protective effect of antibodies [27]. Another study demonstrated that the antibody titers were correlated th protection against COVID-19 [28]. Considering that the cellular immune response is suppressed in cancer tients, even adequate antibody levels may not effectively protect from the infection. Based on this, the application additional doses, especially in cancer patients, may come to the fore in light of future studies. Patients receiving tive chemotherapy and those in older age groups might be among the priority groups.

Another finding of our study was that the control group reported side effects more frequently, especially after t first dose. The reason might be that cancer patients experience such side effects due to the disease itself and th treatment processes, even before vaccination. The frequency of side effects reported after the second dose was fou to be similar in both groups; this can be explained by the decrease in the perception of the side effects followi the second dose.

Finally, when we created two groups by matching the patient and control groups by age and gender, the significa difference in seropositivity rates between the groups persisted.

This study had some limitations. First, we measured only spike IgG antibody levels of the participants but did n assess neutralizing antibody levels. However, studies have shown that neutralizing antibody levels are correlated wi spike IgG antibody levels [29]. Second, we did not evaluate the pre-vaccination antibody levels of the participan Nevertheless, we excluded patients who had a documented COVID-19 infection at any time before enrollment

The median follow-up period after vaccination was 3 months, and eight patients were infected with COVID- during this period. The patient group will be followed up for long-term results to evaluate the effect of vaccinati and antibody levels on disease prevention.

Conclusion

This study highlighted the efficacy and safety of CoronaVac in cancer patients. The seropositivity rate was low in cancer patients than in non-cancer controls, especially in patients aged over 60 years and those receiving acti chemotherapy. Further studies with larger sample sizes are needed to determine the effective vaccine type a vaccine dose for cancer patients so that cancer patients might be protected from COVID-19-related morbidity a mortality without disrupting their oncological treatments.

Summary points

- COVID-19 is associated with high morbidity and mortality in cancer patients, but there are limited data on the efficacy and safety of currently used COVID-19 vaccines in cancer patients.
- We compared the seropositivity rate of cancer patients with non-cancer controls after CoronaVac administratior and evaluated the factors affecting seropositivity in cancer patients.
- 776 cancer patients and 715 non-cancer volunteers who received a second dose of inactivated vaccine in 4–6 weeks were included in the study.
- The seropositivity rate and antibody levels were significantly lower in the patient group than in the non-cancer controls ($p < 0.001$). Age and chemotherapy were associated with lower seropositivity in cancer patients ($p < 0.001$).
- Side effects reported after the first dose were significantly higher in the control group ($p = 0.001$). There was no significant difference between the two groups after the second dose.
- The high seropositivity rate of cancer patients indicates that these patients benefit from the vaccine as protectio from COVID-19 infection.
- It should be kept in mind that patients over the age of 60 and receiving chemotherapy have lower seropositivity rates and are in a higher risk group for COVID-19.

Financial & competing interests disclosure

This study was funded by the Oncological Clinical Research Association (ONKAD). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval (02/28) and have followed the princip outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Zhu N, Zhang D, Wang W *et al.* A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.* 382(8), 727–7 (2020).

Sun L, Warner JL, Parikh RB. Immune responses to SARS-CoV-2 among patients with cancer: what can seropositivity tell us? *JAMA Oncol.* 7(8), 1123–1125 (2021).

Comment about seropositivity in cancer patients.

WHO. WHO coronavirus (COVID-19) dashboard. https://covid19.who.int/region/euro/country/tr

Liang W, Guan W, Chen R *et al.* Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. *Lancet Oncol.* 21(3), 335–337 (2020).

Robilotti EV, Babady NE, Mead PA *et al.* Determinants of COVID-19 disease severity in patients with cancer. *Nat. Med.* 26(8), 1218–1223 (2020).

Rugge M, Zorzi M, Guzzinati S. SARS-CoV-2 infection in the Italian Veneto region: adverse outcomes in patients with cancer. *Nat. Cancer* 1(8), 784–788 (2020).

Saini KS, Tagliamento M, Lambertini M *et al.* Mortality in patients with cancer and coronavirus disease 2019: a systematic review and pooled analysis of 52 studies. *Eur. J. Cancer* 139, 43–50 (2020).

Bakouny Z, Hawley JE, Choueiri TK *et al.* COVID-19 and cancer: current challenges and perspectives. *Cancer Cell* 38(5), 629–646 (2020).

Stöss C, Steffani M, Pergolini I *et al.* Impact of the COVID-19 pandemic on surgical oncology in Europe: results of a European survey. *Dig. Surg.* 38(4), 259–265 (2021).

Riera R, Bagattini ÂM, Pacheco RL, Pachito DV, Roitberg F, Ilbawi A. Delays and disruptions in cancer health care due to COVID-19 pandemic: systematic review. *JCO Glob. Oncol.* 7, 311–323 (2021).

Lai AG, Pasea L, Banerjee A *et al.* Estimated impact of the COVID-19 pandemic on cancer services and excess 1-year mortality in people with cancer and multimorbidity: near real-time data on cancer care, cancer deaths and a population-based cohort study. *BMJ Open* 10(11), e043828 (2020).

Fillmore NR, La J, Szalat RE *et al.* Prevalence and outcome of COVID-19 infection in cancer patients: a National Veterans Affairs study. *J. Natl Cancer Inst.* 113(6), 691–698 (2021).

Kuderer NM, Choueiri TK, Shah DP *et al.* Clinical impact of COVID-19 on patients with cancer (CCC19): a cohort study. *Lancet* 395(10241), 1907–1918 (2020).

Haradaa G, Antonacio FF, Gongora AB *et al.* SARS-CoV-2 testing for asymptomatic adult cancer patients before initiating systemic treatments: a systematic review. *Ecancermedicalscience* 14, 1100 (2020).

National Comprehensive Cancer Network. Recommendations of the NCCN COVID-19 vaccination advisory committee. www.nccn.org/docs/default-source/covid-19/2021_covid-19_vaccination_guidance_v3-0.pdf?sfvrsn=b483da2b_60

Solodky ML, Galvez C, Russias B *et al.* Lower detection rates of SARS-COV2 antibodies in cancer patients versus health care workers after symptomatic COVID-19. *Ann. Oncol.* 31(8), 1087–1088 (2020).

Study which evaluated antibody development in cancer patients after COVID-19 infection.

Xia S, Zhang Y, Wang Y *et al.* Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomized, double-blind, placebo-controlled, Phase 1/2 trial. *Lancet Infect. Dis.* 21(1), 39–51 (2021).

Tanriover MD, Doğanay HL, Akova M *et al.* Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomized, placebo-controlled, Phase 3 trial in Turkey. *Lancet* 398(10296), 213–222 (2021).

Study which evaluated the efficacy and safety profile of CoronaVac.

Polack FP, Thomas SJ, Kitchin N *et al.* Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N. Engl. J. Med.* 383(27), 2603–2615 (2020).

Ariamanesh M, Porouhan P, Peyroshabany B *et al.* Immunogenicity and safety of the inactivated SARS-CoV-2 vaccine (BBIBP-CorV) in patients with malignancy. *Cancer Invest.* doi:10.1080/07357907.2021.1992420 1–9 (2021) (Epub ahead of print).

Study which evaluated the efficacy and safety profile of CoronaVac in cancer patients.

Massarweh A, Eliakim-Raz N, Stemmer A *et al.* Evaluation of seropositivity following BNT162b2 messenger RNA vaccination for SARS-CoV-2 in patients undergoing treatment for cancer. *JAMA Oncol.* 7(8), 1133–1140 (2021).

Study which evaluated the mRNA vaccine BNT162b2 in cancer patients.

Abbott. SARS-CoV-2-Immnuoassays. www.corelaboratory.abbott/int/en/offerings/segments/infectious-disease/sars-cov-2-

Abbott Laboratories. AdviseDx SARS-CoV-2 IgG II package insert (2021). www.fda.gov/media/146371/download

Siber GR, Weitzman SA, Aisenberg AC, Weinstein HJ, Schiffman G. Impaired antibody response to pneumococcal vaccine after treatment for Hodgkin's disease. *N. Engl. J. Med.* 299(9), 442–448 (1978).

Vilar-Compte D, Cornejo P, Valle-Salinas A *et al.* Influenza vaccination in patients with breast cancer: a case–series analysis. *Med. Sci. Monit.* 12(8), CR332–CR336 (2006).

Krammer F. SARS-CoV-2 vaccines in development. *Nature* 586(7830), 516–527 (2020).

27. Harvey RA, Rassen JA, Kabelac CA *et al.* Association of SARS-CoV-2 seropositive antibody test with risk of future infection. *JAMA Intern. Med.* 181(5), 672–679 (2021).

28. Jin P, Li J, Pan H, Wu Y, Zhu F. Immunological surrogate endpoints of COVID-2019 vaccines: the evidence we have versus the evidence we need. *Signal Transduct. Target. Ther.* 6(1), 48 (2021).

29. Sahin U, Muik A, Derhovanessian E *et al.* COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Natu* 586(7830), 594–599 (2020).

4.6. Pacientes com doenças reumáticas autoimunes que já tiveram Covid-19 podem estar protegidos com uma única dose de CoronaVac, sugere estudo

Um estudo publicado na revista The Lancet Rheumatology mostrou que uma única dose de CoronaVac, vacina do Butantan e da Sinovac, pode ser suficiente para promover uma resposta imune robusta em pacientes com doenças reumáticas autoimunes que foram previamente infectados pelo SARS-CoV-2. A pesquisa foi conduzida no Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (FMUSP).

De acordo com a pesquisa, 95% dos 157 pacientes que já tinham contraído Covid-19 e foram imunizados com a CoronaVac produziram uma quantidade média expressiva de anticorpos IgG após a primeira dose. Após a segunda dose, o indicador saltou para 98% dos voluntários.

Os pesquisadores também analisaram 471 indivíduos com doenças reumáticas que nunca tinham tido contato com o coronavírus. A imunização completa com as duas doses da vacina nesse grupo induziu a produção de anticorpos em 75% dos participantes.

Participaram do estudo 1.193 pacientes e 492 controles. Após seleção aleatória de amostras, foram analisadas 942 pessoas (157 com sorologia positiva e 471 com sorologia negativa). Ambos os grupos também contaram, cada um, com 157 indivíduos controles.

Os pesquisadores coletaram amostras sanguíneas dos voluntários imediatamente antes da primeira dose (dia zero), antes da segunda dose (dia 28) e decorridos 69 dias da primeira dose (ou 40 dias da segunda dose).

Memória imunológica

Os resultados do artigo da USP apoiam outras pesquisas feitas com indivíduos com doenças reumáticas autoimunes com sorologia positiva e negativa para Covid-19, que mostram que vacinas de RNA mensageiro e adenovírus induzem o mesmo padrão de resposta imune observado no estudo com a CoronaVac. "Um possível mecanismo que explica essa resposta robusta em quem já teve Covid-19 está relacionado às células B de memória pré-existentes, porque a exposição recorrente é conhecida por gerar respostas mais extensas do que uma infecção primária", apontam os autores do artigo.

Publicado em: 3/12/2021

Immunogenicity and safety of two doses of the CoronaVac SARS-CoV-2 vaccine in SARS-CoV-2 seropositive and seronegative patients with autoimmune rheumatic diseases in Brazil: a subgroup analysis of a phase 4 prospective study

Nadia E Aikawa, Leonard V K Kupa, Sandra G Pasoto, Ana C Medeiros-Ribeiro, Emily F N Yuki, Carla G S Saad, Tatiana Pedrosa, Ricardo Fuller, Samuel K Shinjo, Percival D Sampaio-Barros, Danieli C O Andrade, Rosa M R Pereira, Luciana P C Seguro, Juliana M L Valim, Filipe Waridel, Ana Marli C Sartori, Alberto J S Duarte, Leila Antonangelo, Ester C Sabino, Paulo Rossi Menezes, Esper G Kallas, Clovis A Silva, Eloisa Bonfa

Summary

Background We aimed to examine the immunogenicity pattern induced by the inactivated SARS-CoV-2 vaccine CoronaVac (Sinovac Life Sciences, Beijing, China) in SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases compared with seropositive controls, seronegative patients with autoimmune rheumatic diseases, and seronegative controls.

Methods CoronavRheum is an ongoing, prospective, controlled, phase 4 study, in which patients aged 18 years or older with autoimmune rheumatic diseases, and healthy controls were recruited from a single site (Rheumatology Division of Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo) in São Paulo, Brazil. Participants were vaccinated with two doses of CoronaVac (intramuscular injection, 3 μg in 0·5 mL of β-propiolactone inactivated SARS-CoV-2) on day 0 and on day 28. Blood samples were taken pre-vaccination on day 0, day 28, and also on day 69. For this subgroup analysis, participants were defined as being SARS-CoV-2 seropositive or seronegative prevaccination via anti-SARS-CoV-2 spike (S)1 or S2 IgG (cutoff of 15·0 arbitrary units [AU] per mL) or neutralising antibody titres (cutoff of ≥30%) and were matched for age and sex, via convenience sampling, in a 1:3:1:1 ratio (seropositive patients to seronegative patients to seropositive controls to seronegative controls). The primary outcomes were rates of anti-SARS-CoV-2 S1 and S2 IgG seropositivity and SARS-CoV-2 neutralising antibody positivity at day 28 and day 69 and immunogenicity dynamics assessed by geometric mean titres (GMTs) of IgG and median neutralising activity in seropositive patients with autoimmune rheumatic diseases compared with seronegative patients and seropositive and seronegative controls. We assessed safety in all participants randomly selected for this subgroup analysis. This study is registered with ClinicalTrials.gov, NCT04754698, and is ongoing for long-term immunogenicity evaluation.

Findings Between Feb 4 and Feb 8, 2021, 1418 patients and 542 controls were recruited, of whom 1685 received two vaccinations (1193 patients and 492 controls). After random sampling, our immunogenicity analysis population comprised 942 participants, of whom 157 were SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases, 157 were seropositive controls, 471 were seronegative patients, and 157 were seronegative controls; the median age was 48 years (IQR 38–56) and 594 (63%) were female and 348 (37%) were male. For seropositive patients and controls, an increase in anti-SARS-CoV-2 S1 and S2 IgG titres (seropositive patients GMT 52·3 [95% CI 42·9–63·9] at day 0 *vs* 128·9 [105·6–157·4] at day 28; seropositive controls 53·3 [45·4–62·5] at day 0 *vs* 202·0 [174·8–233·4] at day 28) and neutralising antibody activity (seropositive patients 59% [IQR 39–83] at day 0 *vs* 82% [54–96] at day 28; seropositive controls 58% [41–79] at day 0 *vs* 92% [79–96] at day 28), was observed from day 0 to day 28, without further increases from day 28 to day 69 (at day 69 seropositive patients' GMT was 137·1 [116·2–161·9] and neutralising antibody activity was 79% [57–94]); and seropositive controls' GMT was 188·6 [167·4–212·6] and neutralising antibody activity was 92% [75–96]). By contrast, for seronegative patients and controls, the second dose was required for maximum response at day 69, which was lower in seronegative patients than in seronegative controls. GMTs in seronegative patients were 2·3 (95% CI 2·2–2·3) at day 0, 5·7 (5·1–6·4) at day 28, and 29·6 (26·4–33·3) at day 69, and in seronegative controls were 2·3 (2·1–2·5) at day 0, 10·6 (8·7–13·1) at day 28, and 71·7 (63·5–81·0) at day 69; neutralising antibody activity in seronegative patients was 15% (IQR 15–15) on day 0, 15% (15–15) at day 28, and 39% (15–65) at day 69, and in seronegative controls was 15% (15–15) at day 0, 24% (15–37) at day 28, and 61% (37–79) at day 69. Neither seronegative patients nor seronegative controls reached the GMT or antibody activity levels of seropositive patients at day 69.

Interpretation By contrast with seronegative patients with autoimmune rheumatic diseases, seropositive patients have a robust response after a single dose of CoronaVac. Our findings raise the possibility that the reduced immunogenicity observed in seronegative patients might not be the optimum response potential to SARS-CoV-2 vaccination, and therefore emphasise the importance of at least a single booster vaccination in these patients.

Lancet Rheumatol 2021

Published Online December 3, 2021 https://doi.org/10.1016/S2665-9913(21)00327-1

See Online/Comment https://doi.org/10.1016/S2665-9913(21)00392-1

For the Portuguese translation see Online for appendix 1

Rheumatology Division (N E Aikawa PhD, L V K Kupa PhD, S G Pasoto PhD, A C Medeiros-Ribeiro PhD, E F N Yuki PhD, C G S Saad PhD, T Pedrosa PhD, R Fuller PhD, S K Shinjo PhD, P D Sampaio-Barros PhD, D C O Andrade PhD, Prof R M R Pereira PhD, L P C Seguro PhD, J M L Valim MD, F Waridel, Prof C A Silva PhD, Prof E Bonfa PhD), **Pediatric Rheumatology Unit, Instituto da Criança e do Adolescente** (N E Aikawa, Prof C A Silva), **Department of Infectious and Parasitic Diseases** (A M C Sartori PhD, Prof E G Kallas), **and Central Laboratory Division** (Prof A J S Duarte, L Antonangelo PhD), **Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Instituto de Medicina Tropical** (E C Sabino PhD), **and Department of Preventive Medicine** (Prof P R Menezes), **Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil**

Correspondence to: Prof Eloisa Bonfa, Rheumatology Division, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo 01246-903, Brazil **eloisa.bonfa@hc.fm.usp.br**

Funding Fundação de Amparo à Pesquisa do Estado de São Paulo, Conselho Nacional de Desenvolvimento Científico e Tecnológico, and B3-Bolsa de Valores do Brasil.

Introduction

In June, 2021, WHO recommended the emergency use of the inactivated SARS-CoV-2 vaccine, CoronaVac (Sinovac Life Sciences, Beijing, China),[1] which has shown a high level of protection against COVID-19-related hospitalisation and death in the Chilean population.[2] As of Aug 1, 2021, only a quarter of the Brazilian population had received two doses of a SARS-CoV-2 vaccine and CoronaVac accounted for approximately 38% of all SARS-CoV-2 vaccines administered in Brazil.[3]

Previously, we have shown a seroconversion rate of 70·4% with two doses of CoronaVac in adults with autoimmune rheumatic diseases without previous SARS-CoV-2 infection, compared with 95·5% in controls, with a diminished frequency of COVID-19 incident cases after vaccination.[4]

New SARS-CoV-2 variants are emerging and vaccine supply is still restricted worldwide. Therefore, improving strategies to maximise vaccine coverage and enhance immunogenicity is crucial, especially in immunosuppressed populations. A few recent reports, including some preprints, have shown that antibody responses to the first dose of mRNA-based SARS-CoV-2 vaccines in people with previous laboratory-confirmed SARS-CoV-2 infection were similar to or exceeded those found in individuals without previous infection after the second dose,[5–10] raising the possibility of allocating vaccine to other at-risk groups.

However, data are scarce on immune responses to SARS-CoV-2 vaccines in the context of previous SARS-CoV-2 infection in patients with autoimmune rheumatic diseases; a population known to have reduced virus clearance and to be prone to genomic evolution.[11] It is crucial to investigate whether immunogenicity of previous SARS-CoV-2 infection in this population might surpass that of patients without previous SARS-CoV-2 infection who have received two doses, or if humoral response will be limited by an intrinsic defect of these patients' immune system or immunosuppressive treatment, as previously described.[12,13] A study in patients with autoimmune diseases showed that a single dose of mRNA-based or adenovirus-based SARS-CoV-2 vaccine in those with previous SARS-CoV-2 infection could elicit antibody responses similar to two vaccine doses in patients without previous infection, with seroconversion in the vast majority of patients on any immunosuppressive treatment.[14] However, the small sample size of the seropositive group, heterogeneous schedules for blood collection, and the absence of serial samples hampered a definitive conclusion on the kinetics of humoral response.[14] Understanding antibody kinetics is even more relevant in the context of the approval of a third

Research in context

Evidence before this study

Pre-existing immunity for COVID-19 affects vaccine response and might allow a change in the current vaccination guidelines, allowing for increased vaccine availability. We searched PubMed for publications between Dec 1, 2020, and Aug 27, 2021, for studies published in English on COVID-19 vaccines in patients with autoimmune rheumatic disease, using the terms "seropositive" AND ("vaccination" OR "vaccine") AND ("COVID-19" OR "SARS-CoV-2") AND ("autoimmune" OR "rheumatic"). Few reports suggested that one dose of mRNA-based SARS-CoV-2 vaccine could elicit a large antibody response in SARS-CoV-2 seropositive individuals, with no further increase in antibody response after the second dose. However, we found no studies with data for inactivated SARS-CoV-2 vaccines and little information on patients with autoimmune rheumatic diseases, in whom immunogenicity is known to be reduced. Moreover, only few studies have focused on immunological analysis of neutralising antibodies, which are relevant in immune protection against SARS-CoV-2 infection.

Added value of this study

This study provides the first evidence that previous exposure to SARS-CoV-2, independent of symptoms, in patients with autoimmune rheumatic diseases results in distinct dynamics of antibody response (measured via anti-SARS-CoV-2 spike antibody titres and neutralising antibody activity) to an inactivated SARS-CoV-2 vaccine (CoronaVac; Sinovac Life Sciences, Beijing, China) compared with patients without previous exposure. Our study expands on previous reports in healthy individuals and a small sample of seropositive patients with autoimmune rheumatic diseases immunised with mRNA-based or adenovirus-based SARS-CoV-2 vaccines, in that seropositive patients showed a robust boost in antibody response after the first dose of inactivated vaccine, independent of their underlying disease or treatment. No further increase in response was observed between the first and second dose, and the antibody response remained up to 6 weeks after the second dose.

Implications of all the available evidence

The CoronaVac vaccine presents distinct kinetics of immune response in seropositive patients with autoimmune rheumatic diseases compared to seronegative patients. Our finding raises the possibility that the reduced immunogenicity observed in seronegative patients might not represent the optimum response potential and suggest that these patients might benefit from booster doses.

SARS-CoV-2 vaccine dose for immunocompromised individuals in some countries.[15]

To add to this knowledge, we assessed the dynamics of antibody production induced by the inactivated CoronaVac vaccine in patients with autoimmune rheumatic disease who were SARS-CoV-2 seropositive and those who were SARS-CoV-2 seronegative compared with SARS-CoV-2 seropositive and seronegative controls.

Methods

Study design and participants

This is a retrospective subgroup analysis of a large ongoing prospective, controlled, phase 4 study (CoronavRheum) of immunogenicity and safety of two doses of the inactivated SARS-CoV-2 vaccine CoronaVac in patients with autoimmune rheumatic diseases[4] being conducted in a single site (Rheumatology Division of Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo) in São Paulo, Brazil, to assess the dynamics of response to this SARS-CoV-2 inactivated vaccine in patients with autoimmune rheumatic diseases who are seropositive for SARS-CoV-2-specific antibodies at baseline compared with those who are seronegative at baseline and with controls.

For the main trial, patients with autoimmune rheumatic diseases from our outpatient rheumatology clinics in São Paulo, Brazil, were consecutively invited to participate in the study if they were aged 18 years or older and if they fulfilled the classification criteria for one of the following autoimmune rheumatic diseases: rheumatoid arthritis, systemic lupus erythematosus, spondiloarthritis, vasculitis, primary Sjogren's syndrome, systemic sclerosis, systemic autoimmune myopathies, and primary antiphospholipid syndrome. Additionally, hospital services workers, health professionals, and hospital administrative service employees or their relatives without autoimmune rheumatic disease and not taking immunosuppressive therapy were recruited to comprise the healthy control group. Exclusion criteria were in accordance to our previous report.[4] Key exclusion criteria were history of anaphylactic response to vaccine components, acute febrile illness or symptoms compatible with COVID-19 at vaccination, decompensated heart failure (class III or IV), demyelinating disease, previous vaccination with any SARS-CoV-2 vaccine, history of live virus vaccine up to 4 weeks before enrolment, receipt of inactivated virus vaccine up to 2 weeks before enrolment, patients who were being treated in hospital for any reason, and not providing consent to participate.

The study protocol was approved by the National and Institutional Ethical Committee (CAAE: 42566621.0.0000.0068) and written informed consent was obtained from all participants.

Procedures

The CoronaVac COVID-19 vaccine (batch number 20200412, Sinovac Life Sciences, Beijing, China) used in this study was supplied by the Instituto Butantan (São Paulo, Brazil). Patients and controls were vaccinated in a two-dose schedule, via intramuscular injection with 3 μg of vaccine in 0·5 mL of β-propiolactone inactivated SARS-CoV-2. The first dose and blood collection were done for most participants on Feb 9–10, 2021 (day 0), the second dose with blood collection was done on March 9–10, 2021 (day 28), and the last blood collection was done on April 19, 2021 (day 69) at the hospital convention center. For this subgroup analysis, incident COVID-19 cases were assessed from day 0 to day 79.

Laboratory tests were done at the central laboratory division of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (supervised by AJSD and LS). Human IgG antibodies against the SARS-CoV-2 spike (S) 1 and S2 proteins were measured using a chemiluminescent immunoassay (Indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy). The lower limit of quantification of the assay was 3·8 UA/mL and seropositivity was defined as anti-SARS-CoV-2 (S1/S2) IgG of more than 15·0 UA/mL. For titres below the limit of quantification, a value of 1·9 UA/mL was assigned.

A SARS-CoV-2 neutralising antibody assay was done using the cPass SARS-CoV-2 neutralisation antibodies detection kit (GenScript, Piscataway, NJ, USA). Results are expressed as positive or negative neutralising antibodies according to the manufacturer recommended cutoff of percentage signal inhibition (≥30% inhibition).[16] Medians and IQRs of the percentage of neutralising activity were calculated at all timepoints (at day 0, day 28 and day 69), attributing the value of 15% (half of positive inhibition cutoff) to undetectable levels (<30%).

The study was monitored by independent vaccine experts, who comprised the Data Safety Monitoring Board. Local and systemic vaccine-related adverse effects were carefully reviewed with each participant at in-person visits on day 28 and day 69, as previously reported.[2] Vaccine adverse effect severity was ranked according to WHO definitions.[17] 24 h access to the medical team was available to all participants, including telephone contacts, email, and WhatsApp messages for safety support, from day 0 until day 69.

All participants completed a standardised questionnaire to assess their history of SARS-CoV-2 infection at baseline (appendix 2 p 8). Reports of any previous positive RT-PCR test were requested. Social risk factors associated with increased risk of exposure to SARS-CoV-2 were also registered by all participants. Incident cases were defined as new cases of symptomatic SARS-CoV-2 infection, confirmed with RT-PCR between day 0 and day 79.[4] All positive samples tested at our site were further characterised for variants of concern at the same hospital. RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions, as previously described.[18]

For this subgroup analysis, seronegative and seropositive patients with autoimmune rheumatic disease and

See Online for appendix 2

seropositive and seronegative controls were selected from the main cohort. Patients with pre-vaccination positive COVID-19 serology (ie, anti-S1 or S2 IgG or neutralising antibodies) were classified as being seropositive patients or controls and those with pre-vaccination negative COVID-19 serology were classified as seronegative patients or controls.

Outcomes

The primary outcomes were rates of anti-SARS-CoV-2 S1 and S2 IgG seropositivity and SARS-CoV-2 neutralising antibody positivity at day 28 and day 69 and immunogenicity dynamics were assessed by median neutralising activity (ie, activity of neutralising antibodies) and by geometric mean titres (GMTs) of anti-SARS-CoV-2 S1 and S2 IgG and median neutralising antibody activity in SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases compared with seronegative patients and seropositive and seronegative controls.

Secondary outcomes were the influence of previous (ie, prevaccination) symptomatic versus asymptomatic SARS-CoV-2 infection ascertained by RT-PCR or rapid antigen test on vaccine-induced antibody response, antibody dynamics in patients who had symptomatic SARS-CoV-2 infection within the past 3 months (inclusive) versus more than 3 months previously, and vaccine safety.

Exploratory outcomes were prevalence of RT-PCR positive test results among participants (ie, COVID-19 incident cases), analysis of variants of concern, and analysis of infection severity and of social risk factors associated with exposure to SARS-CoV-2.

We did post-hoc analyses of demographic and disease-specific factors associated with anti-SARS-CoV-2 S1 and S2 IgG seropositivity and neutralising antibody positivity at day 28 in seropositive patients, and comparison of vaccine-induced anti-SARS-CoV-2 antibody seropositivity between previously asymptomatic patients and seronegative patients.

Statistical analysis

All treatment groups in this subgroup analysis were selected via convenience sampling from the large phase 4 prospective cohort CoronavRheum.[4] Seronegative and seropositive patients with autoimmune rheumatic disease and seropositive and seronegative controls were selected from the main cohort, in a 1:3:1:1 ratio, matched for age (up to 5 years difference) and sex using an in-house program run on Excel (Microsoft 2018) for random selection of individuals in each category.

We present categorical variables as n (%), continuous variables as median (IQR), and anti-SARS-CoV-2 S1 and S2 IgG serology titres as geometric means (95% CI). We did statistical comparisons between groups using the χ^2 test or Fisher's exact test for categorical variables and Student's *t* test or the Mann-Whitney *U* test for continuous variables. We transformed anti-SARS-CoV-2 S1 and S2 IgG titre data in natural logarithm(ln) before analysis, and we describe the values of ln(IgG) titres and neutralising antibodies according to groups (seropositive and seronegative patients with autoimmune rheumatic diseases and seropositive and seronegative controls) and at each assessment timepoint (day 0, day 28, and day 69). We compared ln-transformed anti-SARS-CoV-2 S1 or S2 IgG titres and neutralising antibody activity between groups and between timepoints (day 0, day 28, and day 69) using generalised estimating equations with normal marginal distribution (for IgG titres) and gamma distribution (for neutralising antibodies) and identified binding function assuming first order autoregressive correlation matrix between time-points. We did Bonferroni multiple comparisons to identify differences between groups and timepoints.

The primary outcomes and post-hoc analysis of factors associated with anti-SARS-CoV-2 S1 and S2 IgG seropositivity and neutralising antibody positivity at day 28 were assessed in all participants who were selected as part of random sampling. Secondary outcomes were assessed in all participants who received vaccine, before random sapling. We assessed incident case surveillance in all participants of CoronavaRheum of data cutoff (April 29, 2021) from day 0 to day 79. Participants with RT-PCR-confirmed previous SARS-CoV-2 infection between day 0 and day 69 were excluded from the immunogenicity analyses, but were included in incident case surveillance (from day 0 to day 79).

We assessed vaccine safety among all the participants who were randomly selected for this subgroup analysis. We did this by analysing reports of any vaccine side-effect and the reviewing the standardised diary completed by the participants, including local and systemic manifestations. Vaccine-related adverse effects were carefully reviewed with each participant at in-person visits on day 28 and day 69.

We did all analyses using the IBM-SPSS for Windows (version 22.0) and we made graphs of mean profiles and SEs using the Microsoft-Excel 2010 software. The tests were performed with a significance level of 5%. This study is registered with ClinicalTrials.gov, NCT04754698

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Instituto Butantan supplied the study product and had no other role in the trial.

Results

Between Feb 4 and Feb 8, 2021, 1418 patients and 542 controls were recruited to CoronavRheum, of whom 1193 patients and 492 controls attended three study visits that occurred on Feb 9–10, 2021 (day 0), on March 9–10, 2021 (day 28), and on April 19, 2021 (day 69), and received two doses of inactivated SARS-CoV-2 vaccine on days 0 and 28. Of the 1685 participants who received both doses of CoronaVac, 86 were excluded from further analyses because they became infected with SARS-CoV-2 during the

study or did not have available data for analysis (figure 1). After applying the exclusion criteria and random sampling, the final study groups for this immunogenicity analysis comprised 942 participants, of whom 157 were SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases, 157 were seropositive controls, 471 were seronegative patients with autoimmune rheumatic diseases, and 157 were seronegative controls (figure 1).

In the analysable population, the median age was 48 years (IQR 38–56) and 594 (63%) were female and 348 (37%) were male. Participant groups were comparable with regards to baseline age, sex, and ethnicity distribution (table 1) . A shorter disease duration was observed in SARS-CoV-2 seropositive patients with autoimmune rheumatic disease than in seronegative patients (p=0·011; table 1). Disease and treatment distributions were similar between seropositive and seronegative patients (table 1).

A high proportion of seropositive patients and controls had anti-SARS-CoV-2 S1 or S2 IgG seropositivity at day 28 (149 [95%] of 157 *vs* 155 [99%] of 157; p=0·10) and these proportions remained high at day 69 (154 [98%] *vs* 157 [100%]; p=0·25) with comparable seropositivity rates at both timepoints (table 2). In the seropositive patient and control groups we also observed high proportions of participants with neutralising antibody positivity at day 28 (138 [88%] *vs* 151 [96%]; p=0·0067), which was sustained at day 69 (141 [90%] *vs* 155 [99%]; p=0·0005); although, a lower proportion of patients were neutralising antibody positive than controls.

A distinct pattern was detected for seronegative patients with autoimmune rheumatic diseases, with a low proportion of patients having anti-SARS-CoV-2 S1 or S2 IgG seropositivity (99 [21%] of 471) and neutralising antibody positivity (108 [23%]) at day 28, and the second dose was required to obtain moderate proportions with

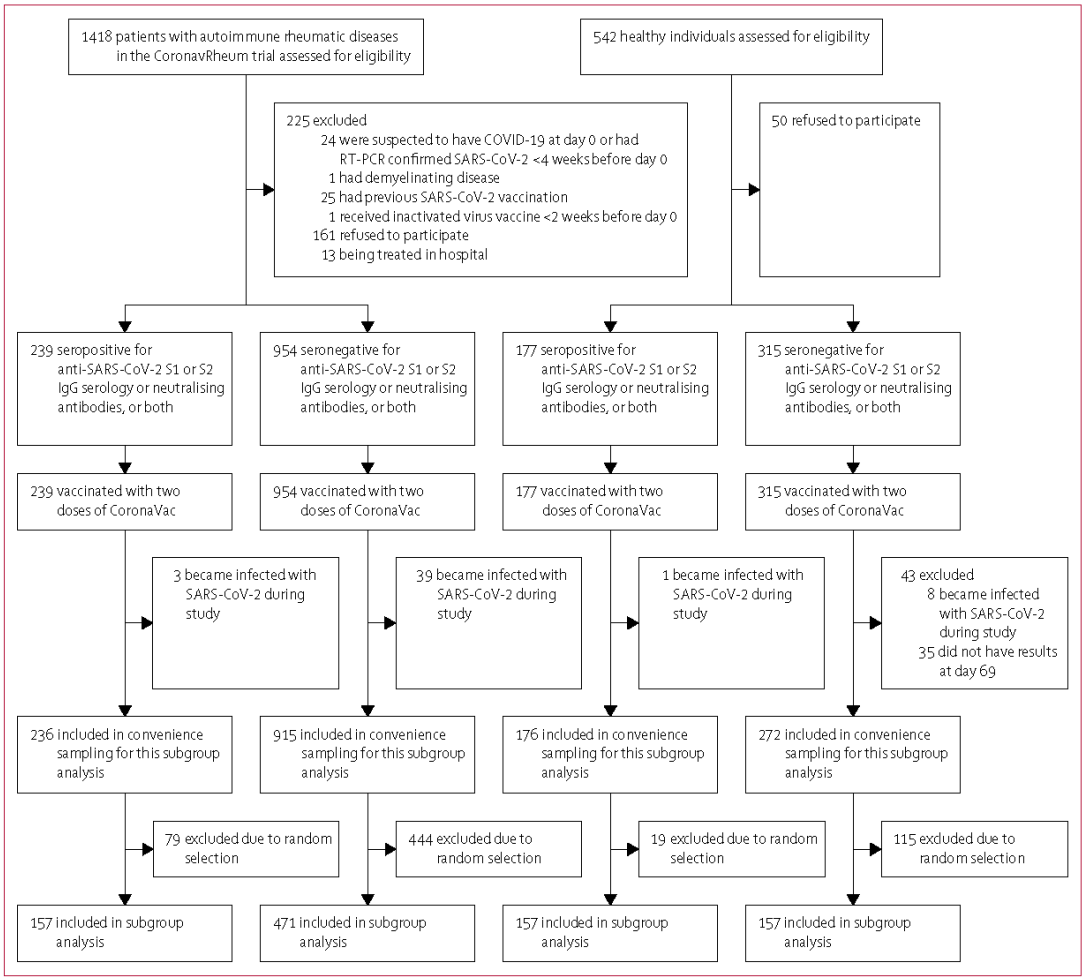


Figure 1: **Study profile**
S=spike.

	SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases (n=157)	SARS-CoV-2 seronegative patients with autoimmune rheumatic diseases (n=471)	SARS-CoV-2 seropositive controls (n=157)	SARS-CoV-2 seronegative controls (n=157)	p value
Demographic data					
Age, years					
Median	48 (38–57)	48 (38–56)	48 (36–56)	48 (38–57)	0·98
>65	4 (3%)	12 (3%)	4 (3%)	7 (4%)	>0·999
At diagnosis	33 (22–43)	30 (22–40)	..	..	0·11
Disease duration, years	12 (7–19)	14 (8–22)	..	..	0·011
Sex	..	..	..	..	>0·999
Female	99 (63%)	297 (63%)	99 (63%)	99 (63%)	..
Male	58 (37%)	174 (37%)	58 (37%)	58 (37%)	..
Race	..	..	..	..	0·12
White	78 (50%)	234 (50%)	58 (37%)	76 (48%)	..
African-Latin American	76 (48%)	226 (48%)	95 (61%)	74 (47%)	..
Asian	1 (1%)	7 (1%)	4 (3%)	4 (3%)	..
Indigenous Brazilian	2 (1%)	4 (1%)	0	3 (2%)	..
Clinical data					
Autoimmune rheumatic disease					
Rheumatoid arthritis	39 (25%)	125 (27%)	..	..	0·68
Axial spondyloarthritis	32 (20%)	80 (17%)	..	..	0·34
Psoriatic arthritis	16 (10%)	56 (12%)	..	..	0·56
Systemic lupus erythematosus	37 (24%)	115 (24%)	..	..	0·83
Systemic vasculitis	10 (6%)	32 (7%)	..	..	0·85
Systemic autoimmune myopathy	6 (4%)	20 (4%)	..	..	>0·999
Systemic sclerosis	7 (4%)	13 (3%)	..	..	0·29
Primary Sjögren's syndrome	6 (4%)	16 (3%)	..	..	0·80
Primary antiphospholipid syndrome	4 (3%)	13 (3%)	..	..	>0·999
Current therapies					
Hydroxychloroquine	44 (28%)	127 (27%)	..	..	0·80
Sulfasalazine	20 (13%)	45 (10%)	..	..	0·26
Prednisone	47 (30%)	182 (39%)	..	..	0·050
Dose, mg per day	6 (5–10)	5 (5–10)	..	..	0·21
Immunosuppressive drugs	94 (60%)*	296 (63%)	..	..	0·51
Methotrexate	44 (28%)	135 (29%)	..	..	0·88
Leflunomide	18 (11%)	57 (12%)	..	..	0·83
Mycophenolate mofetil	16 (10%)	55 (12%)	..	..	0·61
Azathioprine	15 (10%)	49 (10%)	..	..	0·76
Other†	8 (5%)	19 (4%)	..	..	0·57
Biologic agent	53 (34%)	174 (37%)	..	..	0·47
TNF inhibitor	27 (17%)	81 (17%)	..	..	>0·999
Abatacept	5 (3%)	20 (4%)	..	..	0·56
Secukinumab	11 (7%)	21 (4%)	..	..	0·21
Other‡	10 (6%)	49 (10%)	..	..	0·13

Data are n (%) or median (IQR). p values are calculated using data across all groups where possible, and only between the seropositive and seronegative patients for rheumatic disease characteristics. Categorical variables were compared between groups using the χ^2 test or Fisher's exact test and all continuous variables were compared using the Mann-Whitney *U*. *Sums to more than the patient numbers provided because seven patients were taking more than one immunosuppresive drug. †Cyclophosphamide, cyclosporin, tacrolimus, and tofacitinib. ‡Tocilizumab, rituximab, belimumab, and ustekinumab.

Table 1: **Baseline demographic and clinical characteristics of SARS-CoV-2 seropositive and seronegative patients with autoimmune rheumatic diseases and seropositive and seronegative controls**

anti-SARS-CoV-2 S1 or S2 IgG seropositivity (353 [75%]) and neutralising antibody positivity (289 [61%]) at day 69. Likewise, seronegative controls also needed two doses to reach a moderate response at day 69 (proportion with IgG seropositivity was 57 [36%] of 157 at day 28 and 150 [96%] at day 69; neutralising antibody positivity was

56 [36%] at day 28 and 128 [82%] at day 69; table 2). The proportion of seronegative patients who had a response was significantly lower than among seropositive patients at day 28 (p<0·0001) and day 69 (p<0·0001). Also, the proportion of seronegative controls with IgG seropositivity and neutralising antibody positivity was lower than among seropositive patients at day 28 (p<0·0001) but not at day 69 (p=0·34), and the proportion who had neutralising antibody positivity was lower at day 28 (p<0·0001) and day 69 (p=0·036; table 2).

Seropositive patients and controls had similar vaccine-induced antibody dynamics, with substantial increases from day 0 to day 28 and no further increase from day 28 to day 69 (table 3, figure 2; appendix 2 pp 2–3).

We observed changes from day 0 to day 28 in seronegative patients for anti-SARS-CoV-2 S1 or S2 IgG GMTs (from 2·3 arbitrary units [AU]/mL [95% CI 2·2–2·3] to 5·7 [5·1–6·4]; table 3, figure 2 [data presented as ln(IgG)]) and for neutralising antibody activity (15% [IQR 15–15] to 15% [15–15]; table 3; appendix 2 pp 2–3). A substantial increase was seen in anti-SARS-CoV-2 S1 or S2 IgG GMTs from day 28 to day 69 for seronegative patients (from 5·7AU/mL [95% CI 5·1–5·4] to 29·6 AU/mL [26·4–33·3]). A similar increase was observed for neutralising antibody activity from day 28 to day 69 (15% [IQR 15–15] to 39% [15–65]; table 3; appendix 2 pp 1–2). Seronegative controls had a similar pattern, with minor increases after the first dose and substantial increases after the second dose for both anti-SARS-CoV-2 S1 or S2 IgG GMTs and neutralising antibody activity (table 3; appendix 2 pp 1–2). Significantly lower proportions of seronegative patients had IgG seropositivity and neutralising antibody positivity at day 28 and day 69 than did seronegative controls (table 2).

In line with these findings, when the groups were compared at different timepoints, seropositive patients and controls had similar IgG titres at day 0 (p>0·999) and day 69 (p=0·41) but titres were higher in seropositive controls at day 28 (p=0·0080; table 3). For neutralising antibody activity, the values were similar at day 0 (p>0·999), day 28 (p=0·119), and day 69 (p=0·300; table 3). By contrast, seropositive patients had significantly higher values than seronegative patients at all timepoints for IgG GMTs and neutralising antibody activity (table 3). Seropositive patients also had significantly higher IgG GMTs and neutralising antibody activity than did seronegative controls at all timepoints (table 3; appendix 2 pp 1–2).

In a post-hoc analysis, we found no significant associations between demographic data and specific autoimmune rheumatic diseases and therapies and anti-SARS-CoV-2 S1 or S2 IgG seropositivity and neutralising antibody positivity in the seropositive patient group at day 28 (appendix 2 p 3).

We assessed the effect of previous symptomatic versus asymptomatic SARS-CoV-2 infection on vaccine-induced response. Of 157 seropositive patients with autoimmune rheumatic diseases, 43 had no confirmation of previous acute infection by RT-PCR or rapid antigen test and therefore they were excluded from this analysis. The remaining 114 patients with a previous symptomatic RT-PCR or rapid antigen test confirmed COVID-19 were included. 41 (36%) of 114 had a previous symptomatic infection and 73 (64%) had a previous asymptomatic infection. We found significantly higher levels anti-SARS-CoV-2 S1 or S2 IgG GMTs on day 0 in the symptomatic group than in the asymptomatic group (75·1 AU/mL [95% CI 55·4–101·8] *vs* 39·0 AU/mL [28·0–54·3]; p=0·010) and thereafter similar levels after each vaccine dose (figure 3A). Neutralising antibody activity responses showed the same pattern, with higher day 0 neutralising activity in the previously symptomatic group than in the previously asymptomatic group (74% [IQR 47–88] *vs* 53% [37–75]; p=0·042) but similar levels at day 28 (p=0·12) and day 69 (p=0·20; figure 3B). At day 69, the comparison of previously asymptomatic patients with seronegative patients revealed significantly higher IgG seropositivity (71 [97%] of 73 *vs* 353 [75%] of 471; p<0·0001) and neutralising antibody positivity (66 [90%] *vs* 289 [61%]; p<0·0001) in previously asymptomatic seropositive patients than in seronegative patients (post hoc). IgG and neutralising antibodies positivities were also higher in previously asymptomatic seropositive patients than in seronegative patients at day 0 (p<0·0001) and at day 28 (p<0·0001; data not shown).

	Anti-SARS-CoV-2 S1 or S2 IgG seropositivity			Neutralising antibody positivity		
	Day 0	Day 28	Day 69	Day 0	Day 28	Day 69
SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases (n=157)	140 (89%)	149 (95%)	154 (98%)	135 (86%)	138 (88%)	141 (90%)
SARS-CoV-2 seropositive controls (n=157)	149 (95%)	155 (99%)	157 (100%)	140 (89%)	151 (96%)	155 (99%)
SARS-CoV-2 seronegative patients with autoimmune rheumatic diseases (n=471)	0	99 (21%)	353 (75%)	0	108 (23%)	289 (61%)
SARS-CoV-2 seronegative controls (n=157)	0	57 (36%)	150 (96%)	0	56 (36%)	128 (82%)
p value						
Seropositive patients vs seropositive controls	0·061	0·10	0·25	0·39	0·0067	0·0005
Seropositive patients vs seronegative patients	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001
Seropositive patients vs seronegative controls	<0·0001	<0·0001	0·34	<0·0001	<0·0001	0·036
Seronegative patients vs seronegative controls	>0·999	<0·0001	<0·0001	>0·999	0·0016	<0·0001

Data are n (%). Positivity for anti-SARS-CoV-2 S1 or S2 IgG was defined as post-vaccination titre of ≥15 AU/mL. Positivity for neutralising antibodies was defined as a neutralising activity ≥30%. Frequencies of seropositivity were compared using the χ^2 test.

Table 2: **Anti-SARS-CoV-2 S1 or S2 IgG and neutralising antibody seropositivity rates at baseline and after the first (day 28) and second (day 69) doses of CoronaVac vaccination**

	Anti-SARS-CoV-2 IgG S1 or S2 IgG GMT, AU/mL (95% CI)			Median neutralising activity of neutralising antibodies, % (IQR)		
	Day 0	Day 28	Day 69	Day 0	Day 28	Day 69
SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases (n=157)	52·3 (42·9–63·9)	128·9 (105·6–157·4)	137·1 (116·2–161·9)	59 (39–83)	82 (54–96)	79 (57–94)
SARS-CoV-2 seropositive controls (n=157)	53·3 (45·4–62·5)	202·0 (174·8–233·4)	188·6 (167·4–212·6)	58 (41–79)	92 (79–96)	92 (75–96)
SARS-CoV-2 seronegative patients with autoimmune rheumatic diseases (n=471)	2·3 (2·2–2·3)	5·7 (5·1–6·4)	29·6 (26·4–33·3)	15 (15–15)	15 (15–15)	39 (15–65)
SARS-CoV-2 seronegative controls (n=157)	2·3 (2·1–2·5)	10·6 (8·7–13·1)	71·7 (63·5–81·0)	15 (15–15)	24 (15–37)	61 (37–79)
p value						
Seropositive patients vs seropositive controls	>0·999	0·0080	0·41	>0·999	0·119	0·300
Seropositive patients vs seronegative patients	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001
Seropositive patients vs seronegative controls	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	0·010
Seronegative patients vs seronegative controls	>0·999	<0·0001	<0·0001	>0·999	<0·0001	<0·0001

Proportion of neutralising activity of neutralising antibodies are expressed as median (IQR) and anti-SARS-CoV-2 S1 or S2 IgG antibody titres are expressed as GMTs with 95% CIs. The minimum possible value for neutralising activity is 15% (attributed for values of <30%). AU=arbitrary units. GMT=geometric mean titre.

Table 3: **Geometric mean titres of anti-SARS-CoV-2 S1 or S2 IgG and median percentage of neutralising activity and before (day 0) and after the first (day 28) and second (day 69) doses of CoronaVac vaccination**

The median of elapsed time after SARS-CoV-2 infection in symptomatic patients was 81 days (IQR 8–395) before vaccination. Antibody dynamics in patients with symptomatic infection less than or equal to 3 months (n=21) and more than 3 months (n=20) before vaccination were similar for IgG GMTs and neutralising antibody activity, with a significant increase from day 0 to day 28 (≤3 months only for IgG [p=0·038]; >3 months both IgG [p<0·0001] and neutralising antibodies [p=0·0040]) with no further increase from day 28 to day 69 (≤3 months: IgG p=0·92 and neutralising antibodies p=0·64; >3 months: IgG p=0·55 and neutralising antibodies p=0·49; data not shown).

The inactivated SARS-CoV-2 vaccine CoronaVac was well tolerated, with only mild adverse events reported (appendix 2 pp 5–6). Most adverse events were reported at higher frequencies among seropositive patients than among seronegative patients and seropositive and seronegative controls, particularly abdominal pain (p=0·026) and tremor (p=0·0040) after the first vaccine dose. After the second dose, vaccine injection erythema (p=0·022) and induration (p=0·023) were also more frequently reported by seropositive patients than the other groups. (appendix 2 p 5–6). Among all participants in CoronavRheum as of data cutoff (April 29, 2021), incident cases of SARS-CoV-2 infection confirmed with RT-PCR from day 0 to day 79 were less often observed in seropositive patients than in seronegative patients (three [1%] of 239 *vs* 39 [4%] 954; p=0·031). Eight cases of SARS-CoV-2 infection were reported between day 38 (10 days after complete vaccination) and day 79 (seven among seronegative patients with autoimmune rheumatic diseases and one in a seropositive patient). Regarding infection severity among these cases, seronegative and seropositive patients had a similar frequency of hospital admissions for COVID-19 (one [33%] of three *vs* five [13%] 39; p=0·378) and mechanical ventilation (one [33%] *vs* zero; p=0·071). SARS-CoV-2 genotyping could not be done for all symptomatic participants because 24 participants could not attend our centre for testing and instead had a PCR test for suspected SARS-CoV-2 infection at an external site. Among the 18 samples analysed for variants of concern, 16 (89%) had the gamma (P.1) variant, one (6%) had the alpha (B.1.1.7) variant, and one (6%) had a distinct variant.

Further analysis of incident RT-PCR-confirmed COVID-19 cases in seronegative patients with and without seroconversion after full vaccination (from 10 days after vaccine second dose to day 79) showed no difference between both groups (six [1%] of 707 *vs* one [<1%] of 247; p=0·68).

In the convenience sampled population, the analysis of social risk factors associated with exposure to SARS-CoV-2 showed that suspected COVID-19 contact in close relatives was significantly higher among seropositive patients (70 [45%] of 157) than among seronegative patients (92 [20%] of 471; p<0·0001) and seronegative controls (33 [21%] of 157; p<0·0001), but similar to among seropositive controls (57 [36%] of 157; p=0·035; appendix 2 p 7). Adherence to social quarantine was lower in seropositive controls (25 [16%]) and seronegative controls (35 [22%]) than among seropositive patients (98 [62%]), whereas use of public transportation was less frequent in patients (86 [55%] of seropositive patients and 221 [47%] of seronegative patients) than among controls (130 [83%] of seropositive controls and 121 [77%] seronegative controls; appendix 2 p 7).

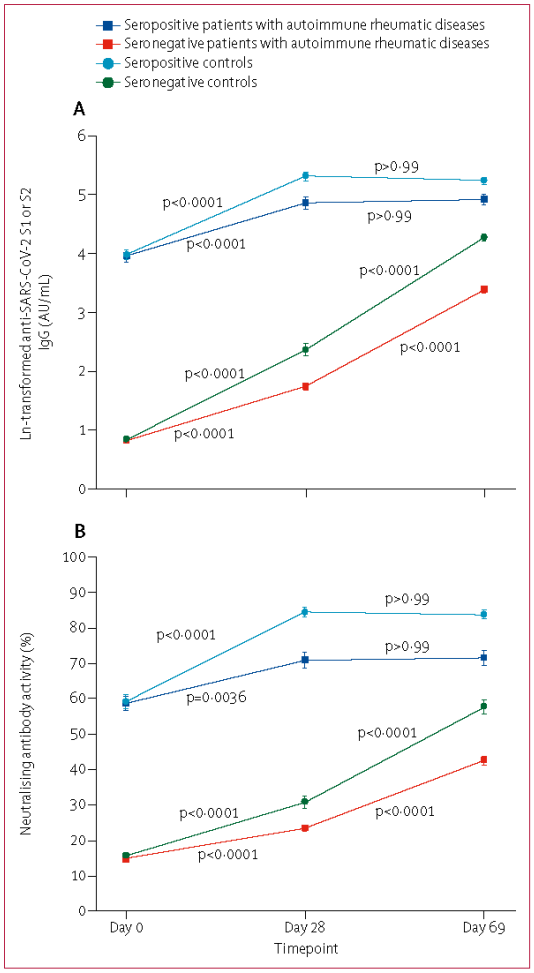


Figure 2: **Anti-SARS-CoV-2 S1 or S2 IgG GMTs (A) and neutralising antibody activity (B) before (day 0) and after the first (day 28) and second (day 69) doses of CoronaVac**

Datapoints are mean values, with error bars showing SD. The minimum possible value for anti-SARS-CoV-2 S1 or S2 IgG is 0·64 (ln 1·9, the value attributed IgG titres of ≤3·8 AU/mL) and for neutralising activity is 15% (attributed for values of <30%). Data are also shown after Bonferroni's multiple comparison in the appendix (pp 2–3). Tests were always two-sided. AU=arbitrary units. GMT=geometric mean titre. S=spike.

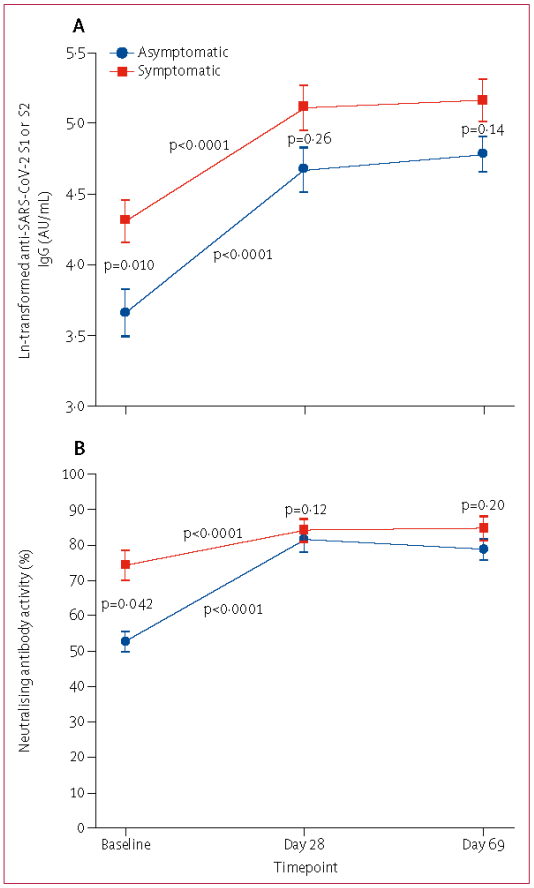


Figure 3: **Anti-SARS-CoV-2 S1 or S2 IgG GMTs (A) and neutralising antibody activity (B) before (day 0) and after the first (day 28) and second (day 69) doses of CoronaVac in seropositive patients with autoimmune rheumatic diseases who had symptomatic infection (n=41) versus asymptomatic infection (n=73)**

Datapoints are means with error bars showing SDs. The minimum possible value for anti-SARS-CoV-2 S1 or S2 IgG is 0·64 (ln 1·9, the value attributed IgG titres of ≤3·8 AU/mL) and for neutralising activity is 15% (attributed for values of <30%). AU=arbitrary units. GMT=geometric mean titre. S=spike.

Discussion

Here we provide the first evidence that previous exposure to SARS-CoV-2, with or without symptoms, results in distinct dynamics of antibody response in a large population of seropositive and seronegative patients with autoimmune rheumatic diseases and controls immunised with an inactivated SARS-CoV-2 vaccine, CoronaVac. Seropositive patients developed a robust response that plateaued between the first and second dose, whereas seronegative patients had moderate antibody production only after two doses of vaccine.

The criterion of positive pre-vaccination immune response that we used, which was independent of symptoms or RT-PCR positivity, offered a broader definition of SARS-CoV-2-exposure.[19] In fact, serological detection is a more precise estimation of previous SARS-CoV-2 infection because asymptomatic infection can account for 40–50% of cases.[20]

Our findings support those of a previous small study in seropositive patients with autoimmune rheumatic diseases showing that mRNA-based and adenovirus-based SARS-CoV-2 vaccines induced high and similar IgG responses, with a substantial increase after the first dose, and no further increase after a second dose.[14] We found here that, in a larger population, the same response occurred with an inactivated vaccine in an immunosuppressed population. The possible underlying mechanism for this robust response is related to pre-existing memory B cells, because recurrent exposure is

known to recall responses to a greater extent than the primary response.[6] In line with these findings, previous reports on an mRNA-based SARS-CoV-2 vaccine have already found that one dose of vaccine was sufficient to increase both cellular and humoral immune responses in healthy individuals who have recovered from COVID-19.[5,7,21,22]

Although patients with autoimmune rheumatic diseases have reduced vaccine immunogenicity, not only to SARS-CoV-2 infection[1,4] but also to other vaccines (eg, for H1N1 influenza),[23] our study provides convincing evidence that patients who have been exposed to SARS-CoV-2 respond adequately to an inactivated SARS-CoV-2 vaccine independent of intrinsic immunological defects or therapy. This finding is of great relevance for individuals who are immunocompromised because the presence of anti-SARS-CoV-2 S1 or S2 antibodies after infection was associated with a considerable reduction of the risk of COVID-19 in health-care workers.[24]

Supporting this result, we observed the same kinetics for neutralising antibody activity in seropositive patients and controls, with a peak reached after the first dose in both groups without further increase after the second dose, and with both groups achieving levels of approximately 70–80%. This immune response in seropositive patients with autoimmune rheumatic disease contrasts with the lower neutralising antibody activity observed in seronegative patients after two doses of same the vaccine[4] and it was also higher than in the seronegative controls. This observation is relevant because of the reported correlation between serum neutralising antibody titres and protection from SARS-CoV-2 infection in human and animal models.[25] Notably, the mRNA-based vaccine BNT162b2 (BioNTech–Pfizer) elicited an increase in anti-SARS-CoV-2 S1 and S2 antibody response after two doses in seropositive healthy individuals (20 times higher than in seronegative individuals)[5] compared with what we observed after vaccination with CoronaVac after two doses; an approximately five times higher antibody response in seropositive patients and controls than in seronegative patients.

Previous studies in patients with autoimmune rheumatic diseases have shown effects of immunosuppressive therapy on antibody production after inactivated virus-based, mRNA-based, and adenovirus-vector-based SARS-CoV-2 vaccinations.[4,14,26,27] Mycophenolate mofetil, methotrexate, rituximab, and TNF inhibitors had a negative effect on anti-SARS-CoV-2 antibody responses, especially in seronegative populations of patients.[4,26,27] By contrast, immunosuppression might be less relevant in seropositive patients, because we observed no detrimental effect on humoral response with these drugs, although we cannot draw any definitive conclusions because of the small sample of patients who were seronegative at day 28. The longer disease duration in our population of seronegative patients than in our seropositive patient population is probably not clinically important for immunogenicity, because age remained balanced between the groups.

Neutralising antibody activity before vaccination was higher in seropositive patients with RT-PCR-confirmed or serology-confirmed previous infection who were symptomatic than in those who were asymptomatic, in accordance with previous reports that neutralising antibody activity correlates positively with disease severity.[28] However, after the first dose of vaccine, both groups reached a similar peak without further increase after the second dose, suggesting that for seropositive patients, a single dose of vaccine results in a boost to the maximum level of response with CoronaVac, independent of the underlying immunosuppressive condition. However, other investigators have reported that asymptomatic or oligosymptomatic individuals who have been exposed to SARS-CoV-2 but are otherwise healthy had a different response after an mRNA-based vaccine (BNT162b2), with lower antibody responses after two doses than symptomatic individuals.[5]

In line with previous studies that included healthy individuals,[9,10] we found that seropositive patients with autoimmune rheumatic diseases had more vaccine-related adverse events than did seronegative patients, which could be related to exacerbated immunity after vaccination, although more data are needed to define the underlying mechanism.[8,19] Ebinger and colleagues[9] found that previously infected individuals had adverse post-vaccine symptoms more frequently than did individuals who had not been previously infected.

The main strength of our study was its prospective design, with all participants receiving vaccine within 2 days at one site, which enabled an adequate comparison of the kinetics of humoral response between study groups. Moreover, the inclusion of study groups balanced for sex and age, and similar groups of patients with autoimmune rheumatic diseases with regards to the diverse diagnoses allowed a more precise assessment of the specific effect of previous exposure to SARS-CoV-2 on the humoral response pattern in the different groups. SARS-CoV-2 vaccine responses might be affected by the presence of immune-mediated inflammatory diseases, age, and sex.[26] Treatment was also similar in the patient groups, which is relevant because glucocorticoids, immunosuppressives, and biological therapies have been reported to impair SARS-CoV-2 vaccine immunogenicity.[4,27] Additionally, few studies on pre-vaccination SARS-CoV-2-exposed individuals have focused on the detailed immunological analysis of neutralising antibodies;[6,7] the leading candidate for a surrogate marker of protection.[29] Notably, the ELISA kit we used to detect neutralising antibodies does not completely replace the gold standard live-virus neutralisation assay, but a comparison between the two tests revealed 98·2% sensitivity and 69·5% specificity.[30]

Our study limitations include the paucity of assessment of memory B-cell and T-cell responses,

which is relevant to assess the recall of antibody response.[6] Also, we have not assessed the effect of CoronaVac on disease activity, but previous large studies in patients with autoimmune rheumatic diseases reported that disease remains stable after SARS-CoV-2 vaccination.[31] The absence of mRNA vaccination as a comparator is another limitation.

In summary, we found that SARS-CoV-2-exposed patients with autoimmune rheumatic diseases have a robust response that plateaus between the first and second dose of CoronaVac, independent of disease or therapy. Our finding raises the possibility that the reduced immunogenicity observed in seronegative patients might not represent the optimum response potential after a first SARS-CoV-2 vaccination, and therefore emphasises the importance of at least a second dose of vaccine in these patients. Future studies are urgently needed to assess whether a third dose of vaccine would be of additional value regarding clinical protection against COVID-19.

Contributors

NEA, LVKK, SGP, ACM-R, EFNY, CGSS, TP, PRM, EGK, CAS, and EB conceived of and designed the study, participated in data collection and analysis, supervised clinical data management, wrote the manuscript, and revised the manuscript. NEA, LVKK, SGP, ACM-R, EFNY, CGSS, TP, CAS, EB, RF, SKS, PDS-B, DCOA, RMRP, LPCS, JMLV, and FW collected epidemiological and clinical data and assisted with the identification of SARS-CoV-2 infection and follow-up of patients. NEA, LVKK, and ACM-R verified the data and had access to raw data. NEA, LVKK, SGP, ACM-R, EFNY, CGSS, CAS, and EB had final responsibility for the decision to submit for publication. AMCS organised and supervised the vaccination protocol, ECS did the SARS-CoV-2 genotyping of positive RT-PCR samples. AJSD and LA supervised the processing of serum samples, SARS-CoV-2 specific antibody ELISAs and neutralisation assays, and SARS-CoV-2 RT-PCRs. All authors helped to edit the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Anonymised participant-level data will be made available on request directed to the corresponding author. Proposals will be reviewed by the Hospital das Clínicas da Universidade de São Paulo review board and, after approval, data can be shared via email in line with the policy and procedures available online. If access to clinical and serological results are requested, approval will be needed from the Hospital das Clínicas da Universidade de São Paulo review board and the National Research Ethics Council and a Material Transfer Agreement in place.

Acknowledgments

We thank Antonio José Rodrigues Pereira, Solange Fusco, and Priscila Tagliaferro Rojo for the organisation of the infrastructure for blood sample collection and vaccination stations. We also thank the Registry Division, Security Division, IT Division, Pharmacy Division of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, and all the volunteers participating in the study. This study was sponsored by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (number 2015/03756-4 to NEA, SGP, CAS, and EB; number 2019/17272-0 to LVKK; number 2017/14352-7 to TP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (305242/2019-9 to EB, 304984/2020-5 to CAS, 305556/2017-7 to RMRP, and 303379/2018-9 to SKS), and B3-Bolsa de Valores do Brasil. Instituto Butantan supplied the vaccine and had no other role in the trial.

References

1 WHO. WHO validates Sinovac COVID-19 vaccine for emergency use and issues interim policy recommendations. Geneva: World Health Organization, June 1, 2021. https://www.who.int/news/item/01-06-2021-who-validates-sinovac-covid-19-vaccine-for-emergency-use-and-issues-interim-policy-recommendations (accessed July 6, 2021).

2 Jara A, Undurraga EA, González C, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. *N Engl J Med* 2021; **385:** 875–84.

3 Brazilian Ministry of Health. COVID-19 vaccination applied doses. https://covid.saude.gov.br/ (accessed June 9, 2021; in Portuguese).

4 Medeiros-Ribeiro AC, Aikawa NE, Saad CGS, et al. Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial. *Nat Med* 2021; **27:** 1744–51.

5 Levi R, Azzolini E, Pozzi C, et al. One dose of SARS-CoV-2 vaccine exponentially increases antibodies in individuals who have recovered from symptomatic COVID-19. *J Clin Invest* 2021; **131:** e149154.

6 Goel RR, Apostolidis SA, Painter MM, et al. Distinct antibody and memory B cell responses in SARS-CoV-2 naïve and recovered individuals following mRNA vaccination. *Sci Immunol* 2021; **6:** eabi6950.

7 Mazzoni A, Di Lauria N, Maggi L, et al. First-dose mRNA vaccination is sufficient to reactivate immunological memory to SARS-CoV-2 in subjects who have recovered from COVID-19. *J Clin Invest* 2021; **131:** e149150.

8 Bradley T, Grundberg E, CODIEFY study team, Selvarangan R. Antibody responses boosted in seropositive healthcare workers after single dose of SARS-CoV-2 mRNA vaccine. *medRxiv* 2021; published online Feb 5. https://doi.org/10.1101/2021.02.03.21251078 (preprint).

9 Ebinger JE, Fert-Bober J, Printsev I, et al. Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2. *Nat Med* 2021; **27:** 981–84.

10 Krammer F, Srivastava K, Alshammary H, et al. Antibody responses in seropositive persons after a single dose of SARS-CoV-2 mRNA vaccine. *N Engl J Med* 2021; **384:** 1372–74.

11 Choi B, Choudhary MC, Regan J, et al. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. *N Engl J Med* 2020; **383:** 2291–93.

12 Grainger R, Machado PM, Robinson PC. Novel coronavirus disease-2019 (COVID-19) in people with rheumatic disease: epidemiology and outcomes. *Best Pract Res Clin Rheumatol* 2021; **35:** 101657.

13 Gianfrancesco M, Hyrich KL, Al-Adely S, et al. Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Ann Rheum Dis* 2020; **79:** 859–66.

14 Boekel L, Steenhuis M, Hooijberg F, et al. Antibody development after COVID-19 vaccination in patients with autoimmune diseases in the Netherlands: a substudy of data from two prospective cohort studies. *Lancet Rheumatol* 2021; **3:** e778–88.

15 US Food and Drug Administration. Coronavirus (COVID-19) update: FDA authorizes additional vaccine dose for certain immunocompromised individuals. Aug 12, 2021. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-additional-vaccine-dose-certain-immunocompromised (accessed Aug 14, 2021).

16 Taylor SC, Hurst B, Charlton CL, et al. A new SARS-CoV-2 dual-purpose serology test: highly accurate infection tracing and neutralizing antibody response detection. *J Clin Microbiol* 2021; **59:** e2438–20.

17 WHO. World alliance for patient safety: WHO draft guidelines for adverse event reporting and learning systems: from information to action. Geneva: World Health Organization, 2005. https://apps.who.int/iris/handle/10665/69797 (accessed Oct 28, 2021).

18 Vogels CBF, Breban MI, Ott IM, et al. Multiplex qPCR discriminates variants of concern to enhance global surveillance of SARS-CoV-2. *PLoS Biol* 2021; **19:** e3001236.

19 Lai CC, Wang JH, Hsueh PR. Population-based seroprevalence surveys of anti-SARS-CoV-2 antibody: an up-to-date review. *Int J Infect Dis* 2020; **101:** 314–22.

20 Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection : a narrative review. *Ann Intern Med* 2020; **173:** 362–67.

21 Gazit S, Shlezinger R, Perez G, et al. Comparing SARS-CoV-2 natural immunity to vaccine-induced immunity: reinfections versus breakthrough infections. *medRxiv* 2021; published online Aug 25. https://doi.org/10.1101/2021.08.24.21262415 (preprint).

For **policy and procedures** see https://www.hc.fm.usp.br/index.php?option=com_content&view=article&id=243:comissao-de-etica-para-analise-de-projetos-de-pesquisa-do-hcfmusp&catid=23&Itemid=229

22 Anichini G, Terrosi C, Gandolfo C, et al. SARS-CoV-2 antibody response in persons with past natural infection. *N Engl J Med* 2021; **385:** 90–92.

23 Saad CGS, Borba EF, Aikawa NE, et al. Immunogenicity and safety of the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune rheumatic diseases. *Ann Rheum Dis* 2011; **70:** 1068–73.

24 Lumley SF, O'Donnell D, Stoesser NE, et al. Antibody status and incidence of SARS-CoV-2 infection in health care workers. *N Engl J Med* 2021; **384:** 533–40.

25 Addetia A, Crawford KHD, Dingens A, et al. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. *J Clin Microbiol* 2020; **58:** e02107–20.

26 Simon D, Tascilar K, Fagni F, et al. SARS-CoV-2 vaccination responses in untreated, conventionally treated and anticytokine-treated patients with immune-mediated inflammatory diseases. *Ann Rheum Dis* 2021; **80:** 1312–16.

27 Boyarsky BJ, Ruddy JA, Connolly CM, et al. Antibody response to a single dose of SARS-CoV-2 mRNA vaccine in patients with rheumatic and musculoskeletal diseases. *Ann Rheum Dis* 2021; **80:** 1098–99.

28 Chvatal-Medina M, Mendez-Cortina Y, Patiño PJ, Velilla PA, Rugeles MT. Antibody responses in COVID-19: a review. *Front Immunol* 2021; published online April 15. https://doi.org/10.3389/fimmu.2021.633184.

29 Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021; **27:** 1205–11.

30 Müller K, Girl P, von Buttlar H, Dobler G, Wölfel R. Comparison of two commercial surrogate ELISAs to detect a neutralising antibody response to SARS-CoV-2. *J Virol Methods* 2021; **292:** 114122.

31 Furer V, Eviatar T, Zisman D, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in adult patients with autoimmune inflammatory rheumatic diseases and in the general population: a multicentre study. *Ann Rheum Dis* 2021; **80:** 1330–38.

4.7. CoronaVac produz anticorpos em 87% dos pacientes com hepatite B, mostra estudo chinês

A CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, gera alta proteção contra a Covid-19 em pacientes que convivem com a hepatite B sem causar reações adversas graves. A conclusão faz parte de um estudo publicado por pesquisadores chineses em artigo na revista Cellular & Molecular Immunology, do grupo Nature. Segundo a pesquisa, após receber a segunda dose do imunizante os pacientes apresentaram uma taxa de soroconversão de 87,25% para anticorpos IgG, e de 74,5% para os anticorpos neutralizantes.

O trabalho Safety and immunogenicity of a SARS-CoV-2 inactivated vaccine in patients with chronic hepatitis B virus infection foi realizado por pesquisadores da Faculdade de Medicina da Universidade Huazhong de Ciência e Tecnologia, de Wuhan, na China, onde eclodiu a pandemia de Covid-19.

Participaram do estudo 284 pacientes com infecção crônica de hepatite B, sendo que 81 deles não haviam sido vacinados, 54 haviam tomado apenas a primeira dose da vacina, e 149 haviam completado o esquema vacinal de duas doses. Decorrido um mês após a primeira ou segunda dose, amostras de plasma foram coletadas e comparadas com as amostras dos não vacinados.

Enquanto a soropositividade para os anticorpos IgG e os anticorpos neutralizantes foi de 87,25% e 74,5%, respectivamente, os dados de reações adversas mostraram que quase todas foram leves, sendo que o sintoma mais comum foi dor no local da injeção seguida por sonolência. Apenas um paciente relatou febre no primeiro dia após a vacinação. Não foram observadas reações adversas graves mesmo nos 20 pacientes com casos mais sérios de infecção crônica de hepatite B (níveis anormais de alanina aminotransferase) ou nos dez pacientes com cirrose hepática.

Este é o primeiro estudo detalhado que analisa a segurança e imunogenicidade da CoronaVac em pacientes com infecção crônica de hepatite B. Estudos anteriores mostraram um risco aumentado de progressão para doença grave em pessoas com cirrose infectadas pelo vírus SARS-CoV-2.

Publicado em: 15/11/2021

CMI

www.nature.com/cmi

CORRESPONDENCE OPEN

Check for updates

Safety and immunogenicity of a SARS-CoV-2 inactivated vaccine in patients with chronic hepatitis B virus infection

Tiandan Xiang[1,2,3], Boyun Liang[1,2,3], Hua Wang[1,2,3], Xufeng Quan[1,2], Shengsong He[1,2], Helong Zhou[1,2], Yongwen He[1,2], Dongliang Yang[1,2], Baoju Wang[1,2]✉ and Xin Zheng[1,2]✉

Cellular & Molecular Immunology; https://doi.org/10.1038/s41423-021-00795-5

Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2 infection, has become a major global public health threat. Although significant advances have been made in developing and applying different vaccines in clinical trials [1, 2], data are limited on the safety and efficacy of the inactivated vaccine in patients with chronic liver disease [3]. Recent studies have preliminarily described the safety and immunogenicity of SARS-CoV-2 vaccines in patients with nonalcoholic fatty liver disease and in liver transplant recipients [4, 5]. However, to date, there is no detailed information on the SARS-CoV-2 inactivated vaccine in patients with chronic hepatitis B (CHB) infection. It has been reported that CHB patients have impaired immune systems [6]. Hence, whether immunocompromised CHB patients within the different clinical stages can be safely vaccinated with the various types of SARS-CoV-2 vaccines and produce an effective immune response remains unclear. Our study aims to provide a comprehensive analysis from different clinical dimensions to characterize the safety and immunogenicity of SARS-CoV-2 inactivated vaccines (BBIBP-CorV, CoronaVac, or WIBP-CorV) within this specific patient population.

A total of 284 CHB patients who were unvaccinated ($n = 81$) or had completed the first ($n = 54$) or second dose ($n = 149$) of the vaccines were enrolled from March 23, 2021, to September 10, 2021 (Table S1). The median time post-vaccination was 33 (IQR, 24–48) days among the 149 completely vaccinated patients. Safety was evaluated by determined the overall incidence of adverse reactions via a standardized questionnaire. Moreover, plasma samples were examined for IgG antibodies against the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein (anti-S-RBD-IgG) and for neutralizing antibodies (NAbs). The complete methods regarding the study design and the statistical analysis are available in the Supplementary methods section.

The adverse reaction data were first analyzed in 149 completely vaccinated CHB patients. The overall incidence of adverse reactions within 7 days was 30.2% (Table S2), which was similar to that found in the phase 3 trials of CoronaVac in Turkey [2]. The most common side effect was injection-site pain (25.5%, 38/149), followed by drowsiness (3%, 3/149); only one patient reported fever on the first day after vaccination. Almost all of the adverse reactions were mild and self-resolved within a few days after vaccination. Serious side effects were not observed even in 20 CHB patients with abnormal alanine aminotransferase levels [61.5 (43–129) U/L] or 10 patients with compensated liver cirrhosis. The results demonstrated that SARS-CoV-2 inactivated vaccines had a favorable safety profile in CHB patients. Given that previous studies have shown an increased risk of progression to severe disease in COVID-19 patients with cirrhosis [7], the benefit of vaccination in compensated cirrhotic patients still outweighs the vaccine-related risk.

Next, we determined the immunogenicity of CHB patients who completed the two doses of the vaccination regimen. The seropositivity for anti-S-RBD-IgG and NAbs was 87.25% and 74.5%, respectively (Fig. 1A). The anti-S-RBD-IgG seropositivity of CHB vaccine recipients was similar to that in a clinical trial of CoronaVac in Turkey (89.7%) but much higher than the reported recently seropositivity of IgG antibodies to the spike protein (76%) in patients with chronic liver disease [5]. Both anti-S-RBD-IgG and NAb levels increased significantly to a higher level after completing the vaccination regimen (Fig. 1B, C, $P < 0.0001$). This finding indicates that SARS-CoV-2 inactivated vaccines can elicit an optimal antibody response even though some CHB patients may have pre-existing compromised immune function.

The seropositivity and antibody titers in CHB patients were further compared according to sex, age, antiviral therapy, and body mass index stratification (Fig. 1D, E). We found that younger patients (<40 y) had higher seropositivity for anti-S-RBD-IgG ($P < 0.05$), and female patients exhibited increased seropositivity for NAbs ($P < 0.05$). Recent clinical trials have also reported a similar trend: younger individuals and female vaccine recipients exhibited stronger humoral immune responses to vaccination [2]. Interestingly, the patients undergoing nucleos(t)ide analog therapy had a significantly higher NAb titer than those who were not ($P < 0.05$) (Fig. 1D, E). Long-term antiviral therapy can inhibit viral replication and facilitate the restoration of the impaired immune system by recovering the function of circulating dendritic cells, natural killer cells, or T cells, particularly nucleotide analogs that can induce the production of IFN-λ3 [6, 8]. These factors may account for the higher antibody titer in patients with antiviral therapy. Given that nucleos(t)ide analog therapy does not affect vaccine-induced immune responses, it should be continuously administered during vaccination to avoid negatively impacting CHB treatment.

[1]Department of Infectious Diseases, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. [2]Joint International Laboratory of Infection and Immunity, Huazhong University of Science and Technology, Wuhan, China. [3]These authors contributed equally: Tiandan Xiang, Boyun Liang, Hua Wang. ✉email: bjwang73@163.com; xin11@hotmail.com

Received: 22 September 2021 Accepted: 11 October 2021
Published online: 15 November 2021

SPRINGER NATURE

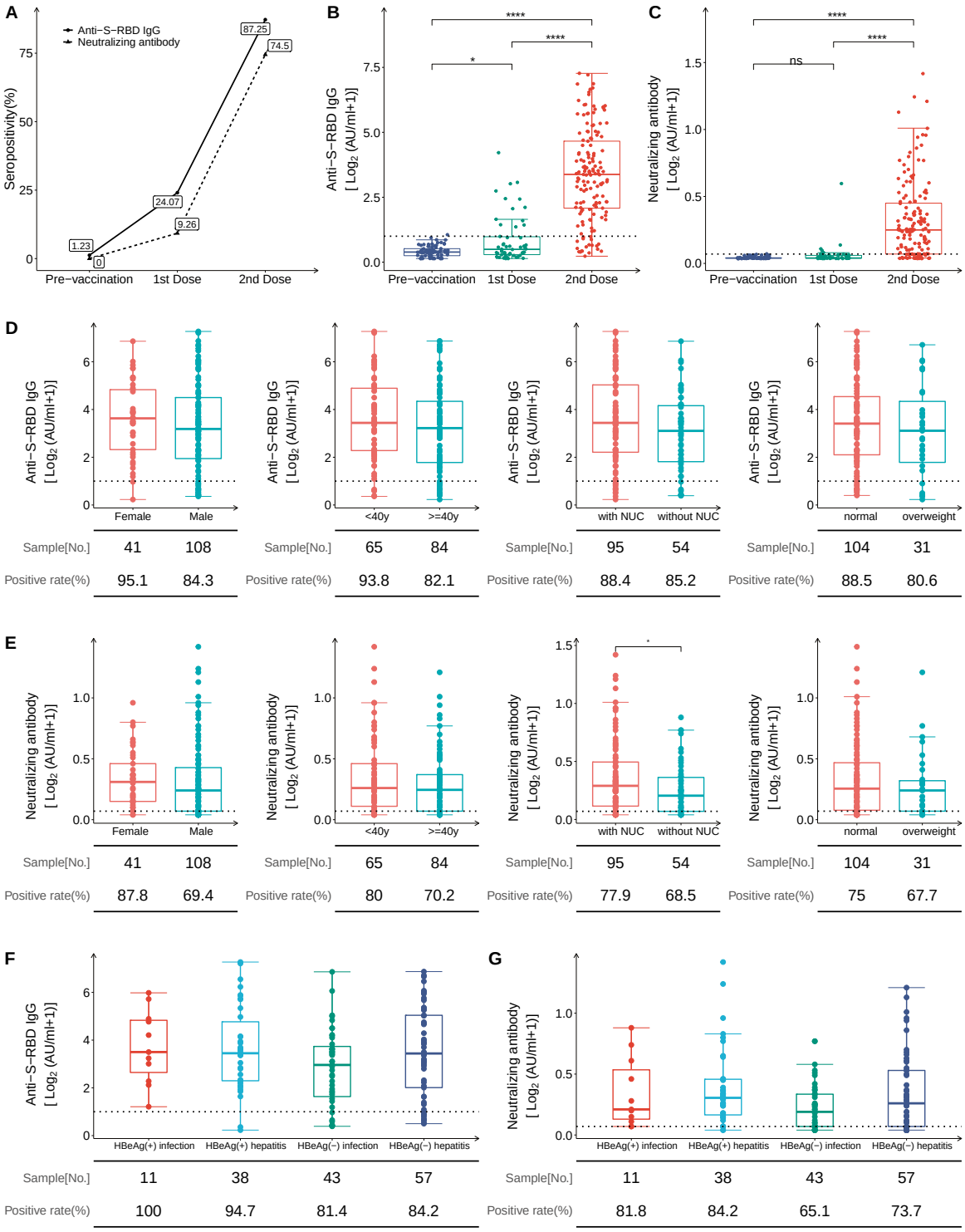


Fig. 1 Antibody responses following immunization with the inactivated vaccine in CHB patients. **A** The seropositivity of anti-S-RBD-IgG and NAbs in CHB patients. **B**, **C** Kinetics of the anti-S-RBD-IgG and NAb titers in vaccine-induced sera at different time points in CHB patients. Prevaccination, $n = 81$; first dose, $n = 54$; second dose, $n = 149$. **D**, **E** The comparison of anti-S-RBD-IgG and NAb titers stratified according to sex, age, nucleos(t)ide analog (NUC) therapy, and BMI (overweight: BMI ≥ 25; 14 patients had unavailable BMI values). **F**, **G** Comparison of anti-S-RBD-IgG (**F**) and NAb titers (**G**) in $HBeAg^+$ chronic infection, $HBeAg^+$ chronic hepatitis, $HBeAg^-$ chronic infection, and $HBeAg^-$ chronic hepatitis individuals [9]. Sample numbers and positive rates are shown underneath. *P* values were determined using a Mann–Whitney U test or a Kruskal–Wallis test followed by Dunn's multiple comparisons test for antibody titers and Fisher's exact test for seropositivity. The horizontal dotted line represents the cutoff value. ns: no significance, $*p < 0.05$, $****p < 0.0001$

Finally, we compared the antibody responses among the CHB patients in the various clinical stages of infection. The CHB participants were divided into four groups according to the "EASL 2017 Clinical Practice Guidelines on the Management of Hepatitis B Virus Infection" [9]: (I) HBeAg-positive chronic HBV infection, (II) HBeAg-positive chronic hepatitis B, (III) HBeAg-negative chronic HBV infection, and (IV) HBeAg-negative chronic hepatitis B. There was no significant difference in seropositivity or antibody titers among the four groups constituting the 149 CHB patients (Fig. 1F, G), suggesting the general applicability of the inactivated vaccines within this patient population.

Altogether, our study reveals that SARS-CoV-2 inactivated vaccines achieve a favorable safety profile and efficient immunogenicity in patients with CHB in real-world vaccination scenarios. The results are encouraging despite some patients not being vaccinated following the standard dose interval time in clinical trials or the two dosages of the inactivated vaccine not being from the same manufacturer.

REFERENCES

1. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020;383:2603–15.
2. Tanriover MD, Doganay HL, Akova M, Guner HR, Azap A, Akhan S, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. Lancet. 2021;398:213–22.
3. Cornberg M, Buti M, Eberhardt CS, Grossi PA, Shouval D. EASL position paper on the use of COVID-19 vaccines in patients with chronic liver diseases, hepatobiliary cancer and liver transplant recipients. J Hepatol. 2021;74:944–51.
4. Wang J, Hou Z, Liu J, Gu Y, Wu Y, Chen Z, et al. Safety and immunogenicity of COVID-19 vaccination in patients with non-alcoholic fatty liver disease (CHESS2101): a multicenter study. J Hepatol. 2021;75: 439-41.
5. Thuluvath PJ, Robarts P, Chauhan M. Analysis of antibody responses after COVID-19 vaccination in liver transplant recipients and those with chronic liver diseases. J Hepatol. 2021; Online ahead of print.
6. Liu N, Liu B, Zhang L, Li H, Chen Z, Luo A, et al. Recovery of circulating CD56(dim) NK cells and the balance of Th17/Treg after nucleoside analog therapy in patients with chronic hepatitis B and low levels of HBsAg. Int Immunopharmacol. 2018;62:59–66.
7. Moon AM, Webb GJ, Aloman C, Armstrong MJ, Cargill T, Dhanasekaran R, et al. High mortality rates for SARS-CoV-2 infection in patients with pre-existing chronic liver disease and cirrhosis: Preliminary results from an international registry. J Hepatol. 2020;73:705–8.
8. Murata K, Asano M, Matsumoto A, Sugiyama M, Nishida N, Tanaka E, et al. Induction of IFN-lambda3 as an additional effect of nucleotide, not nucleoside, analogues: a new potential target for HBV infection. Gut. 2018;67:362–71.
9. EASL. 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67:370–98.

ACKNOWLEDGEMENTS

This study was supported by the Applied Basic and Frontier Technology Research Project of Wuhan (2020020601012233); the Science and Technology Key Project on Novel Coronavirus Pneumonia, Hubei Province (2020FCA002); the Fundamental Research Funds for the Central Universities (2020kfyXGYJ016 and 2020kfyXGYJ028); the National Science and Technology Major Project of China (2018ZX10302206, 2018ZX10723203, and 2017ZX10304402-002-005); the National Key R&D Program of China (2017YFC0908104); and the Innovation Team Project of the Health Commission of Hubei Province (WJ2019C003). The authors thank AiMi Academic Services and Diana Liu for English language editing. We wish to thank the assistance given by Jia Liu, Cheng Peng, Jun Wu, Juan Xu, Xuemei Feng, and Rong Zhang in patients enrollment and sample collection.

AUTHOR CONTRIBUTIONS

XZ, BJW, TDX, and BYL designed and conceived the study; TDX, BYL, and HW performed the experiments; TDX, BYL, HW, XFQ, HLZ, YWH, DLY, BJW, and XZ enrolled patients and acquired the data; BYL and HW analyzed the data and contributed to producing the charts; TDX drafted the manuscript; XZ and BJW revised the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41423-021-00795-5.

Correspondence and requests for materials should be addressed to Baoju Wang or Xin Zheng.

Reprints and permission information is available at http://www.nature.com/reprints

4.8. CoronaVac é segura e imunogênica para pacientes com miopatias autoimunes sistêmicas

Um estudo clínico de fase 4 conduzido pela Faculdade de Medicina da Universidade de São Paulo, publicado na revista científica Rheumatology, apresentou evidências de que a CoronaVac é segura e induz resposta imune em pacientes com miopatias autoimunes sistêmicas. Trata-se de um grupo heterogêneo de doenças sistêmicas raras que acometem principalmente os músculos estriados esqueléticos, podendo também atingir pulmões, coração e trato gastrintestinal.

Seis semanas após completarem o esquema vacinal de duas doses da CoronaVac, os 37 pacientes que participaram da pesquisa apresentaram uma atividade média de neutralização semelhante aos 79 indivíduos controles não imunocomprometidos (57,2% vs. 63%). Já a frequência de produção de anticorpos neutralizantes foi de 51,4% nos pacientes e de 77,2% nos controles.

Em relação à produção de anticorpos IgG, 64,9% dos pacientes apresentaram soroconversão, sendo que a titulação geométrica média de anticorpos IgG ficou em 7,9.

Os autores do estudo destacam que, apesar de apresentarem uma menor imunogenicidade em comparação com pessoas saudáveis, algo esperado em indivíduos imunossuprimidos, os pacientes desenvolveram uma boa resposta ao SARS-CoV-2. Além disso, não foi observado nenhum efeito adverso moderado ou grave, comprovando a segurança da CoronaVac nessa população. A frequência de reações adversas leves foi similar em ambos os grupos.

Durante o acompanhamento, seis indivíduos (três pacientes e três controles) tiveram Covid-19, sendo cinco entre a primeira e a segunda dose e apenas um após a segunda dose. Todos desenvolveram sintomas leves e sem necessidade de hospitalização.

Publicado em: 19/10/2021

Artigo

RHEUMATOLOGY

Rheumatology 2021;00:1–11
https://doi.org/10.1093/rheumatology/keab773
Advance access publication 19 October 2021

Original article

Systemic autoimmune myopathies: a prospective phase 4 controlled trial of an inactivated virus vaccine against SARS-CoV-2

Samuel K. Shinjo[1], Fernando H. C. de Souza[1], Isabela B. P. Borges[1], Alexandre M. dos Santos[1], Renata Miossi[1], Rafael G. Misse[1], Ana C. Medeiros-Ribeiro[1], Carla G. S. Saad[1], Emily F. N. Yuki[1], Sandra G. Pasoto[1], Léonard V. K. Kupa[1], Carina Ceneviva[2], Júlia C. Seraphim[1], Tatiana N. Pedrosa[1], Margarete B. G. Vendramini[1], Clóvis A. Silva[3], Nádia E. Aikawa[1,3] and Eloisa Bonfá[1]

Abstract

Objectives. To evaluate immunogenicity and safety of an inactivated SARS-CoV-2 vaccine in systemic autoimmune myopathies (SAMs) and the possible influence of baseline disease parameters, comorbidities and therapy on immune response.

Methods. This prospective controlled study included 53 patients with SAMs and 106 non-immunocompromised control group (CTRL). All participants received two doses of the Sinovac-CoronaVac vaccine (28-day interval). Immunogenicity was assessed by anti-SARS-CoV-2 S1/S2 IgG seroconversion (SC), anti-S1/S2 IgG geometric mean titre (GMT), factor increase GMT (FI-GMT), neutralizing antibodies (NAb) positivity, and median neutralizing activity after each vaccine dose (D0 and D28) and six weeks after the second dose (D69). Participants with pre-vaccination positive IgG serology and/or NAb and those with RT-PCR confirmed COVID-19 during the protocol were excluded from immunogenicity analysis.

Results. Patients and CTRL had comparable sex ($P>0.99$) and age ($P=0.90$). Immunogenicity of 37 patients and 79 CTRL-naïve participants revealed at D69, a moderate but significantly lower SC (64.9% *vs* 91.1%, $P<0.001$), GMT [7.9 (95%CI 4.7–13.2) *vs* 24.7 (95%CI 30.0–30.5) UA/ml, $P<0.001$] and frequency of NAb (51.4% *vs* 77.2%, $P<0.001$) in SAMs compared with CTRL. Median neutralizing activity was comparable in both groups [57.2% (interquartile range (IQR) 43.4–83.4) *vs* 63.0% (IQR 40.3–80.7), $P=0.808$]. Immunosuppressives were less frequently used among NAb+ patients *vs* NAb- patients (73.7% *vs* 100%, $P=0.046$). Type of SAMs, disease status, other drugs or comorbidities did not influence immunogenicity. Vaccine-related adverse events were mild with similar frequencies in patients and CTRL ($P>0.05$).

Conclusion. Sinovac-CoronaVac is safe and has a moderate short-term immunogenicity in SAMs, but reduced compared with CTRL. We further identified that immunosuppression is associated with diminished NAb positivity.

Trial registration. COVID-19 CoronaVac in Patients With Autoimmune Rheumatic Diseases and HIV/AIDS (CoronavRheum), http://clinicaltrials.gov/ct2/show/NCT04754698

Key words: anti-SARS-CoV-2 vaccine, COVID-19, immunogenicity, myositis, neutralizing antibodies, safety

Rheumatology key messages

- Sinovac-CoronaVac is safe for patients with systemic autoimmune myopathies (SAMs).
- Anti-SARS-CoV-2 S1/S2 IgG seroconversion rates were of moderate effect.
- SAM patients have a moderate NAb response but it is reduced compared to the control group.

[1]Division of Rheumatology, [2]Central Laboratory Division and [3]Pediatric Rheumatology Unit, Childrens' Institute, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, SP, Brazil (BR)

Submitted 24 July 2021; accepted 9 October 2021

Correspondence to: Samuel Katsuyuki Shinjo, Av. Dr. Arnaldo, 455, 3° andar, sala 3184, Cerqueira César, CEP 01246-903, Sao Paulo, SP, Brazil. E-mail: samuel.shinjo@usp.br

Introduction

Since the first case in Wuhan, China, in December 2019, the novel coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to more than four million deaths and ~220 million confirmed cases worldwide up to August 2021 [1].

Several studies have identified risk factors associated with severe COVID-19, such as cardiovascular diseases and other comorbidities, male gender and age [2–4]. In addition, systemic autoimmune rheumatic diseases patients may have a worse COVID-19 associated prognosis [5, 6], due to the disease-associated immune dysregulation and immunosuppressive drugs.

Among these systemic autoimmune rheumatic diseases, idiopathic inflammatory myopathies or systemic autoimmune myopathies (SAMs) are a group of rare and heterogeneous diseases that affect primarily the striated skeletal muscles, including DM, PM, antisynthetase syndrome (ASSD), immune-mediated necrotizing myopathies (IMNM), inclusion body myositis, neoplasia-associated myositis and myositis-overlap syndromes [7–9]. Other tissues and systems may be also involved, such as skin, heart, joint, lung and gastrointestinal tract [7].

Gupta *et al.* [10] report challenges for SAMs patients in a large descriptive study during the COVID-19 pandemic, particularly health problems attributed to the pandemic, need to increase or facing of obstacles in the acquisition of medicines, hospitalization for disease-related complications, and reduction of physical exercises. More than a half of patients with SAMs had underlying cardiovascular risk factors and frequently required an increase in drug therapy due to worsening in health-related problems during the pandemic, resulting in a high risk for severe COVID-19 infection. Moreover, patients with SAMs are susceptible to general or opportunistic infections [11, 12]. The use of high doses of glucocorticoids and immunosuppressive drugs are potential risk factors associated with these complications [11]. Therefore, in the context of the COVID-19 pandemic, it becomes extremely important to establish strategic measures to protect these patients against SARS-CoV-2.

An extensive and intensive task force around the world has been combating and containing the SARS-CoV-2 through the development of COVID-19 vaccines. There are, however, few studies evaluating safety and immunogenicity after at least one vaccine dose or two shots of the messenger RNA (mRNA) (BioNTech/Pfizer, Moderna or BNT162b2) and Oxford/Astra- Zeneca/ChAdOx1 nCoV-19 anti-SARS-CoV-2 vaccines in systemic autoimmune rheumatic diseases populations, including <20 SAMs patients [13–19]. Our group has recently reported an overall adequate anti-SARS-CoV-2 IgG seroconversion rate (70.4%) with Sinovac-CoronaVac vaccine in 910 naïve adult autoimmune rheumatic diseases patients compared with 182 age and sex-matched subjects' frequencies showing a diminished frequency of COVID-19 incident cases after immunization [20]. However, none of these studies specifically assessed SAMs and its peculiar disease factors and treatment with an age- and sex-balanced population, in order to more accurately define vaccine response in this group of patients.

Therefore, the present study aimed to evaluate the safety and immunogenicity of Sinovac-CoronaVac vaccine in patients with SAMs compared with a control (CTRL) population, as well as to analyse the potential harmful effect of disease parameters, comorbidities and therapy on vaccine-induced antibody response.

Patients and method

Study design

This prospective phase 4 controlled study is within the protocol of a larger phase 4 trial (clinicaltrials.gov #NCT04754698) that assessed the immunogenicity and safety of the Sinovac-CoronaVac COVID-19 vaccine in a large sample of patients with systemic autoimmune rheumatic diseases [20]. The present study was conducted at a single tertiary centre in Sao Paulo (Brazil). The study had three in-person visits that occurred mostly on 9–10 February 2021 (D0—first vaccine dose), on 9–10 March 2021 (D28—second vaccine dose) and on 19 April 2021 (D69). For those unable to attend, we set a 15-day period for the recap.

The study was conducted according to the Declaration of Helsinki and local regulations and was approved by Comissão de Ética para Análise de Projetos de Pesquisa (CAPPesq) and Comissão Nacional de Ética em Pesquisa (CONEP) – the local and national ethical committees, respectively (CAAE: 42566621.0.0000.0068). Written informed consent was obtained from participants before enrolment.

Participants, inclusion and exclusion criteria

SAMs patients

Patients with SAMs from the Inflammatory Myopathy Outpatient Clinics were invited to participate in the study if they were 18 years or older, and if they fulfilled the EULAR/ACR2017 classification criteria for the inflammatory myopathies [8], and patients with ASSD fulfilled the criteria used by Behrens Pinto *et al.* (2020) [21]. All patients with ASSD had a positive anti-Jo-1 antibody.

Exclusion criteria

Exclusion criteria were history of anaphylactic response to vaccine components, acute febrile illness or symptoms compatible to COVID-19 at vaccination, Guillain–Barre syndrome, decompensated heart failure, demyelinating disease, previous vaccination with any SARS-CoV-2 vaccine, history of live virus vaccine up to four weeks before, history of inactivated virus vaccine up to two weeks before vaccination, history of having received blood products up to six months before vaccination,

cancer-associated myopathies, and inflammatory myopathies overlapping syndromes. Participants with pre-vaccination positive COVID-19 anti-S1/S2 IgG serology and/or SARS-CoV-2 cPass virus-neutralization antibodies (NAb) were excluded from immunogenicity analysis. Patients with RT-PCR confirmed COVID-19 infection after the first vaccine dose and during the protocol were excluded from the immunogenicity analysis.

Seventy SAMs patients were initially selected to participate after the review of the last 3-month medical records using an electronic database (Fig. 1). We preferentially selected patients with well-controlled disease to avoid hospitalizations or changes in therapy during the next three months of study. Selection of patients began within three weeks of the initial protocol, immediately after the emergency's approval of the vaccine in Brazil and invitations began after the ethics committee sanction of the trial. Among the invited patients, 17 patients were excluded due to refusal to participate ($n=3$), hospitalization ($n=1$), difficult coming to the hospital in the pre-established dates for vaccination ($n=5$), scheduled to receive rituximab within short period of vaccination ($n=3$) and disease activity ($n=5$). SAMs patients and CTRL+ groups were balanced for age (up to $\pm$ 5 years' difference) and sex, using an Excel program for random selection of individuals in each category, with a 1 SAM : 2 CTRL ratio. Fifty-three patients comprised the study group, and 106 individuals with no autoimmune rheumatic disease or other immunosuppressive condition and without immunosuppressive therapy composed the CTRL group, who were recruited among healthcare workers from our centre. None of them had received the previous anti-SARS-CoV-2 vaccine.

Demographic data, comorbidities, disease activity parameters and treatments

The patients were clinically assessed, and a standardized interview was performed by physicians with expertise in SAMs. The following data were collected: current age, ethnicity, sex, type of SAMs, disease duration, comorbidities (e.g. systemic arterial hypertension, diabetes mellitus, dyslipidaemia, obesity, myocardial infarction, interstitial lung disease and stroke), habits (smoking) and current therapy (e.g. glucocorticoids, immunosuppressive and immunobiological drugs).

The disease status at D0 (first vaccine dose) was assessed using the International Myositis Assessment and Clinical Studies Groups (IMACS) core set measures, which included application of questionnaires based on scores of the Manual Muscle Testing-8 (MMT-8), Myositis Disease Activity Assessment Visual Analogue Scales (MYOACT), HAQ, global assessment of the disease by the physician and by the patient using the Visual Analogue Scale (VAS) [22–24]. The serum levels of creatine phosphokinase (CPK, reference value: 26–192 U/l) were also tested only at the baseline of the protocol (D0).

Vaccination protocol

The vaccination protocol for patients with SAMs and CTRL consisted of a two-dose schedule of the COVID-19 vaccine. The first dose with blood collection was given mostly on 9–10 February 2021 (D0), the second dose with blood collection on 9–10 March 2021 (D28), and the last blood collection occurred on 19 April 2021 (D69). In case of incident COVID-19 between vaccine doses, the second dose was delayed four weeks after the beginning of symptoms. Ready-to-use syringes loaded with CoronaVac (Sinovac Life Sciences, Beijing, China, batch #20200412), that consists of 3 μg in 0.5 ml of β-propiolactone inactivated SARS-CoV-2 (derived from the CN02 strain of SARS-CoV-2 grown in African green monkey kidney cells – Vero 25 cells) with aluminum hydroxide as an adjuvant were administered intramuscularly in the deltoid area.

Immunogenicity evaluation

Primary immunogenicity evaluation included seroconversion rates of total anti-SARS-Cov-2 S1/S2 IgG and presence of NAb at D69. Secondarily, immunogenicity was assessed by anti-S1/S2 IgG seroconversion and presence of NAb at D28 (after vaccine first dose); geometric mean titres of anti-S1/S2 IgG and their factor-increase in GMT (FI-GMT) at D28 and D69; and median (interquartile range) neutralizing activity of NAb at D28 and D69. In order to assess these outcomes, blood samples (20 ml) from all participants were obtained at days D0 (baseline – immediately before first vaccine dose), D28 (immediately before the second dose) and D69 (six weeks after the second dose). Sera were stored in a -70°C freezer.

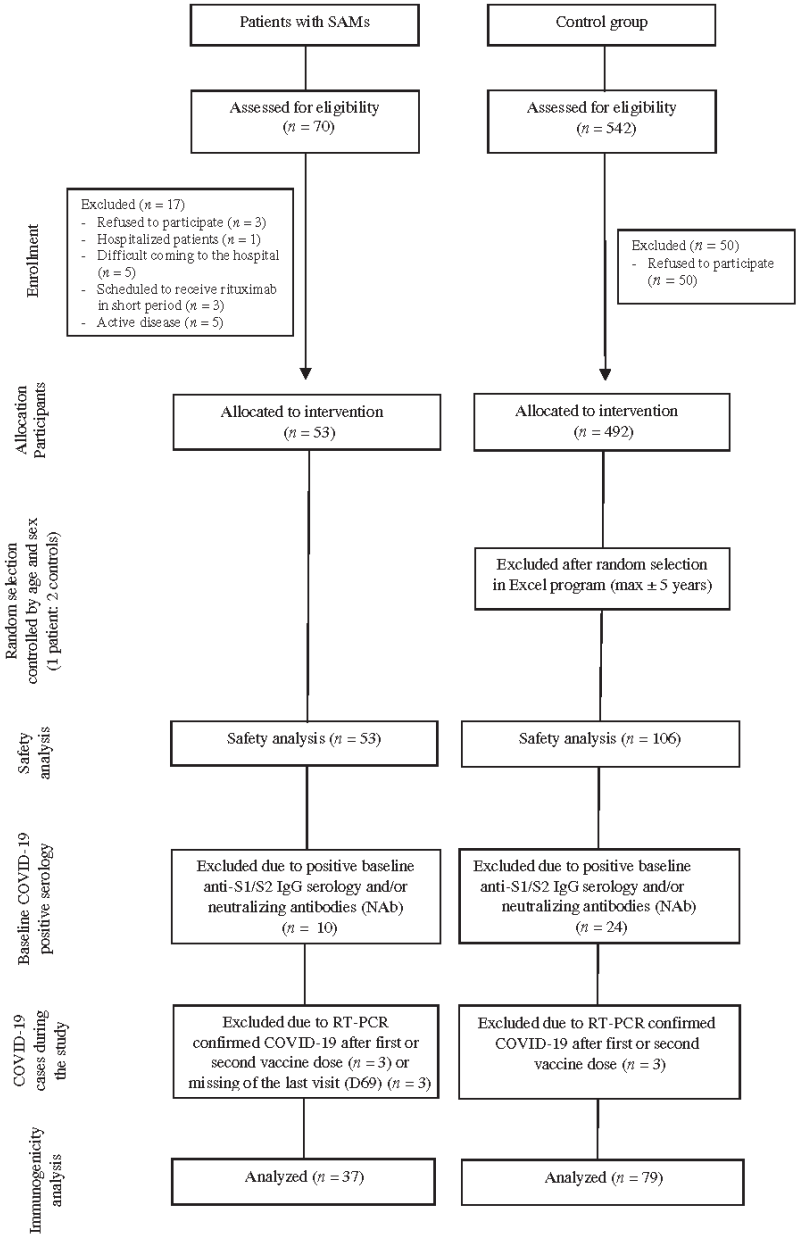
Anti-SARS-CoV-2 S1/S2 IgG antibodies

A chemiluminescent immunoassay was used to measure human IgG antibodies against the S1 and S2 proteins in the RBD (Indirect ELISA, LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy). Seroconversion rate (SC) was defined as positive serology (>15.0 UA/ml) post-vaccination, taking into consideration that only patients with pre-vaccination negative serology were included. Geometric mean titres (GMT) and 95% CI of these antibodies were also calculated at all time points, attributing the value of 1.9 UA/ml (half of the lower limit of quantification 3.8 UA/ml) to undetectable levels (<3.8 UA/ml). The factor increase in GMT (FI-GMT) is the ratio of the GMT after vaccination to the GMT before vaccination, showing the growth in titres. They are also presented and compared as geometric means and 95% CI.

NAb

The SARS-CoV-2 neutralizing antibodies analysis was performed according to manufacturer instructions using sVNT Kit (GenScript, Piscataway, NJ, USA). This analysis detects circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the receptor-binding domain of the viral spike glycoprotein with the angiotensin-converting enzyme 2 cell surface receptor. The tests were performed on the ETI-MAX-

FIG. 1 Flow chart of the present study



Nab: neutralization antibodies; SAMS: systemic autoimmune myopathies.

3000 equipment (DiaSorin, Italy). The samples were classified as either 'positive' (inhibition ≥30%) or 'negative' (inhibition <30%), as suggested by the manufacturer [25]. The frequency of positive samples was calculated at all time points. Median [interquartile range (IQR) 25th–75th] of the percentage of neutralizing activity only for positive samples were calculated at all time points.

Vaccine adverse events and incident cases of COVID-19

Patients and CTRL were advised to report any adverse events of the vaccine and they received on D0 (first dose) and on D28 (second dose) a standardized diary for local and systemic manifestations. Vaccine adverse event severity was defined according to World Health Organization (WHO) definition [1]. Additionally, all

patients and CTRL were instructed to communicate any manifestation associated or not with COVID-19 through telephone, smartphone instant messaging, or email. Independent vaccine experts monitored the study regarding anything adverse for data safety.

RT-PCR for SARS-CoV-2 incident cases

Clinical samples for SARS-CoV-2 RT-PCR consisted of naso- and oropharyngeal swabs, collected at our central laboratory [26] or another laboratory if the patient was unable to come to our hospital.

Statistical analysis

The Kolmogorov–Smirnov test was used to evaluate the distribution of each parameter. The results were presented as mean (S.D.), median (IQR 25th–75th) for continuous variables, whereas the categorical variables were presented as frequency (%). Continuous variables were compared by *t*-Student or Mann–Whitney test for intergroup comparisons when applicable, whereas categorical variables were compared using the χ^2 or Fisher's exact tests when applicable. Specifically, continuous data regarding anti-S1/S2 IgG serology titres are presented as geometric means (95% CI) and compared with the same tests, but in neperian (ln) logarithm-transformed data. Comparisons of ln-transformed IgG titres between SAMs and CTRL in the three time points (D0, D28 and D69) were performed using generalized estimating equations (EEG) with normal marginal distribution and gamma distribution, respectively and identity binding function assuming first-order autoregressive correlation matrix between moments. Results were followed by Bonferroni multiple comparisons to identify differences between groups and time points. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences, version 20.0 (IBM-SPSS for Windows. 20.0, Chicago, IL, USA).

Results

Participants

Fifty-three patients with SAMs (25 with ASSD, 24 with DM and 4 with IMNM) with median disease duration of 6.0 (4.5–9.0) years, and 106 CTRL were prospectively assessed. SAMs and CTRL had comparable current age ($P = 0.925$), female sex ($P > 0.999$) and ethnicity distribution ($P = 0.312$) (Table 1). The disease duration was 6.0 (4.5–9.0) months. Seven (13.2%) patients with SAMs and seven (6.9%) CTRL ($P = 0.166$) were unable to attend on the defined days; therefore, they had up to 15 days for the recap.

Comorbidities were balanced in SAMs and CTRL, except for a higher prevalence of systemic arterial hypertension, dyslipidaemia and obesity in patients with SAMs compared with CTRL (Table 1). Interstitial lung disease occurs only in patients with SAMs, whereas one stroke case occurred in CTRL. There were no cases of arterial or venous thrombosis, chronic kidney disease, pulmonary hypertension, hemorrhage, liver disease, cancer, tuberculosis and HIV in both groups.

All patients had stable or low disease activity, based on the IMACS core set scores at baseline (Table 1). Concerning current treatment, 15 (28.3%) patients were under prednisone with current median dose of 6.3 (5.0–13.8) mg/day and the cumulative dose of the six previous months was 1.6 (1.1–4.8) g. In addition, 44 (83.0%) patients were using immunosuppressive drugs, six (11.3%) patients were under rituximab and one (1.9%) tofacitinib (Table 1). None of the immunosuppressive drugs, including CYC, rituximab and mycophenolate mofetil were discontinued in patients with SAMs.

Vaccine immunogenicity

Samples

For this assessment, 16 patients with SAMs were excluded: 10 patients had pre-vaccination positive COVID-19 IgG serology or NAb positivity, three patients had RT-PCR confirmed COVID-19 after the first dose of vaccine until D69, two patients who did not attend the final visit, and one patient deceased (not related to COVID-19). In the CTRL group, 24 individuals were excluded from immunogenicity analysis for positive anti-S1/S2 IgG and/or NAb at D0 and another three for RT-PCR confirmed COVID-19 during the protocol.

Anti-SARS-CoV-2 IgG antibodies

Humoral response to Sinovac-CoronaVac is shown in Table 2. Analysis of SARS-CoV-2 S1/S2 IgG response revealed that six weeks after vaccine second dose, SC rates were moderate but lower than CTRL (64.9% *vs* 91.1%, respectively; $P < 0.001$). GMT and FI-GMT were also significantly lower in patients with SAMs compared with CTRL ($P < 0.001$ and $P < 0.001$, respectively) (Table 2).

NAb

After complete vaccination, NAb positivity was also moderate but reduced when compared with CTRL (51.4% *vs* 77.2%, $P < 0.01$), whereas the median NAb was comparable in both groups after the first [39.2 (38.4–52.5) *vs* 46.6 (36.9–73.3), $P = 0.573$] and second dose [57.2 (43.4–83.4) *vs* 63.0 (40.3–80.7), $P = 0.808$] (Table 3).

Factors associated with seroconversion and NAb positivity among patients with SAMs

Patients with NAb positivity used less often immunosuppressive drugs than those without NAb (73.7% *vs* 100%, $P = 0.046$). Likewise, the median of patient global activity (VAS) was lower in the former group [1.0 (0.0–3.0) *vs* 2.0 (2.0–3.0), $P = 0.029$] (Table 4), although both groups were characterized by mild value alterations.

Vaccine tolerance and safety

Sinovac-CoronaVac vaccine tolerance and safety analysis is shown in Table 5. No moderate/severe adverse events were observed. The frequency of mild symptoms

Table 1 Baseline characteristics of patients with systemic autoimmune myopathies and controls

	SAMs ($n = 53$)	CTRL ($n = 106$)	*P*-value
Demographics			
Current age (years)	50.7 (11.1)	50.5 (10.6)	0.925
Disease duration (years)	6.0 (4.5–9.0)	–	–
Female sex	40 (75.5)	80 (75.5)	>0.999
White ethnicity	28 (52.8)	47 (44.3)	0.312
Comorbidities and habits			
Systemic arterial hypertension	28 (52.8)	38 (35.8)	0.041
Diabetes mellitus	10 (18.9)	18 (17.0)	0.768
Dyslipidaemia	14 (26.4)	7 (6.6)	0.001
BMI $\geq$30 kg/m^2	26 (49.1)	27 (25.5)	0.003
Myocardial infarction	2 (3.8)	2 (1.9)	0.601
Interstitial lung disease	19 (35.8)	0	–
Stroke	0	1 (0.9)	–
Current smoking	2 (3.8)	11 (10.4)	0.222
Type of diseases			
DM	24 (45.3)	–	–
Antisynthetase syndrome	25 (47.2)	–	–
IMNM	4 (7.5)	–	–
Disease status			
HAQ (0.0–3.0)	0.0 (0.0–0.0)		
Patients' EVA (0–10)	1.0 (0.0–3.0)		
Physician's EVA (0–10)	0.0 (0.0–1.0)		
MMT-8 (0–80)	80 (80–80)		
MYOACT (0–60)	0.0 (0.0–0.0)		
Creatine phosphokinase (U/l)	110 (78–174)		
Current therapy			
Prednisone (current use)	15 (28.3)	–	–
Dose (mg/day)	6.3 (5.0–13.8)		
Cumulative dose[a] (g)	1.6 (1.1–4.8)		
Immunosuppressive drugs	44 (83.0)	–	–
Mycophenolate mofetil	19 (35.8)	–	–
MTX	11 (20.8)	–	–
AZA	8 (15.1)	–	–
LEF	6 (11.3)	–	–
Ciclosporin	3 (5.7)	–	–
CYC	2 (3.8)	–	–
Rituximab	6 (11.3)	–	–
Tofacitinib	1 (1.9)	–	–

Results are expressed in mean (S.D.), median (interquartile range 25th–75th), and *n* (%). CTRL: control group; HAQ: Healthy Assessment Questionnaire; IMNM: immune-mediated necrotizing myopathies; MMT: manual muscle testing; MYOACT: Myositis Disease Activity Assessment Visual Analogue Scales; SAMs: systemic autoimmune myopathies; VAS: Visual Analogue Scale. [a]Last six months.

was comparable in patients with SAMs and CTRL, except for significantly higher prevalence of headache in patients with SAMs at the first vaccine dose (26.4% *vs* 8.5%, $P = 0.002$). No differences were observed in the frequencies of myalgia or muscle weakness among groups.

COVID-19 incident cases

A total of six incident symptomatic cases of COVID-19 confirmed by RT-PCR were identified among SAMs ($n = 3$) and CTRL ($n = 3$) throughout the study period. Three CTRL individuals and two patients with SAMs had COVID-19 between the first and second dose, whereas one patient had COVID-19 three weeks after the second dose. All participants had mild symptoms and none required hospitalization.

Discussion

To our knowledge, this is the largest study demonstrating a short-term disease safety and moderate immunogenicity of anti-SARS-CoV-2 inactivated vaccine in patients with SAMs but reduced compared with an age and sex-balanced non-immunocompromised control group. We further identified that immunosuppressive therapy reduces antibody response.

Table 2 Seroconversion rates and anti-SARS-CoV-2 S1/S2 IgG GMT in naïve patients with myositis and control group

	Before vaccine First dose	After vaccine First dose (D28)			After vaccine Second dose (D69)		
	GMT	SC	GMT	FI-GMT	SC	GMT	FI-GMT
SAMs ($n=37$)	2.1 (1.9–2.3)	3 (8.1)	3.3 (2.5–4.3)[a]	1.5 (1.2–2.0)[a]	24 (64.9)[a]	16.6 (9.7–28.3)[a,b]	7.9 (4.7–13.2)[a]
CTRL ($n=79$)	2.4 (2.1–2.7)	27 (34.2)	9.6 (7.2–12.9)	4.1 (3.2–5.1)	72 (91.1)	58.5 (48.4–70.8)[c,d]	24.7 (20.0–30.5)
P-value (SAMs *vs* CTRL)	0.630	0.005	<0.001	<0.001	<0.001	<0.001	<0.001

Results are expressed in mean (95% CI) or frequency (%). CTRL: control group; FI-GMT: factor increase of geometric mean titres; GMT: geometric mean titres (AU/ml); SAMs: systemic autoimmune myopathies; SC: seroconversion. Frequencies of SC are presented as number (%), and they were compared using two-sided χ^2 test between SAMs and CTRL at D28 and D69. Anti-S1/S2 IgG were expressed as geometric means (CI95%). Titers were compared between SAM and CTRL and between time points (D0, D28 and D69) using generalized estimating equations (EEG) with normal marginal distribution and gamma distribution, respectively. Results were followed by Bonferroni multiple comparisons to identify differences between groups and time points. [a]$P<0.001$ for longitudinal comparison of GMT in SAMs at D69 *vs* baseline. [b]$P<0.001$ for longitudinal comparison of GMT in SAMs at D69 *vs* D28. [c]$P<0.001$ for longitudinal comparison of GMT in controls at D28 and D69 *vs* baseline. [d]$P<0.001$ for longitudinal comparison of GMT in controls at D69 *vs* D28.

Table 3 Neutralizing antibodies and neutralizing activity in naïve patients with myositis in comparison to control group

	After vaccine first dose		After vaccine second dose	
	Subjects with positive NAb	Neutralizing activity (%)	Subjects with positive NAb	Neutralizing activity (%)
SAMs ($n=37$)	5 (13.5)[a]	39.2 (38.4–52.5)	19 (51.4)[a]	57.2 (43.4–83.4)
CTRL ($n=79$)	26 (32.9)	46.6 (36.9–73.3)	61 (77.2)	63.0 (40.3–80.7)

Results are expressed in median (25th–75th) or frequency (%). CTRL: control group; NAb: neutralizing antibodies; SAMs: systemic autoimmune myopathies. [a]$P<0.01$ in comparison to controls.

One advantage of the present study was the prospective analysis with a representative sample of patients with well-defined SAMs taking into consideration that they are a group of patients with rare conditions and the strict exclusion criteria applied herein. Another strength of the present study was that patients had comparable age and sex of the CTRL, as immunogenicity can vary according to these parameters [27, 28]. We also excluded cancer-associated myopathies and other associated autoimmune conditions in order to have a more homogeneous population [29]. A limitation of the present study is the inclusion of patients solely from a tertiary care centre who may not represent the full spectrum of SAMs and could result in an overestimation of the disease or drug complications in the context of a more severe disease.

All individuals were followed with three scheduled face-to-face appointments, telephone calls and smartphone instant messaging, which allowed a precise monitoring of vaccine-induced adverse effects in all phases of the study. The exclusion of pre-vaccination seropositive participants and those with RT-PCR confirmed COVID-19 during the study period were also relevant, allowing a more accurate evaluation of this vaccine response. The strict schedule for blood sample collection and vaccination in two days aimed to guarantee that most patients with SAMs and CTRL would be vaccinated in the same timeframe during the pandemic, precluding the possible confounding nonlinear relationship between the elapsed time and immune response.

Currently, most studies on the immunogenicity and safety of the anti-SARS-CoV-2 vaccines in patients with systemic autoimmune rheumatic diseases evaluated distinct vaccines, mainly mRNA or vector-borne vaccines [13–19]. Regarding safety, all those studies related acceptable rates of adverse events [13–20], without apparent impact on disease activity. However, specifically for SAMs, the number of patients was small [14–19], and they were not evaluated with specific and validated instruments for SAMs. The current study adds data about the safety of the inactivated vaccine in well-controlled patients with SAMs, using specific and validated instruments at baseline [22–24]. Importantly,

TABLE 4 Baseline characteristics of patients regarding to seroconversion for anti-SARS-CoV-2 S1/S2 IgG, and neutralizing antibodies positivity

	Patients with SC ($n = 24$)	Patients without SC ($n = 13$)	*P*-value	Patients with Nab ($n = 19$)	Patients without Nab ($n = 18$)	*P*-value
Demographic data						
Current age (years)	50.0 (11.7)	55.0 (8.9)	0.187	48.8 (11.6)	54.9 (9.4)	0.090
Current age >60 years	3 (12.5)	2 (15.4)	>0.999	2 (10.5)	3 (16.7)	0.660
Female sex	16 (66.7)	12 (92.3)	0.119	13 (68.4)	15 (83.3)	0.447
White ethnicity	14 (58.3)	6 (46.2)	0.478	11 (57.9)	9 (50)	0.630
Diseases						
DM	11 (45.8)	6 (46.2)	>0.999	7 (36.8)	10 (55.6)	0.330
Antisynthetase syndrome	11 (45.8)	6 (46.2)	>0.999	10 (52.6)	7 (38.9)	0.515
IMNM	2 (8.4)	1 (7.6)	>0.999	2 (10.6)	1 (5.5)	>0.999
Disease parameters						
HAQ (0.0–3.0)	0.0 (0.0–1.2)	0.0 (0.0–0.0)	0.537	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.746
Patients' EVA (0–10)	1.0 (0.0–2.8)	3.0 (2.0–3.0)	0.058	1.0 (0.0–3.0)	2.0 (2.0–3.0)	**0.029**
Physician's EVA (0–10)	0.0 (0.0–0.0)	0.0 (0.0–3.0)	0.387	0.0 (0.0–0.0)	0.0 (0.0–3.0)	0.221
MMT-8 (0–80)	80 (80–80)	80 (79–80)	0.353	80 (80–80)	80 (80–80)	0.558
MYOACT (0–60)	0.0 (0.0–10.0)	0.0 (0.0–3.5)	0.479	0.0 (0.0–1.0)	0.0 (0.0–0.8)	0.940
Creatine phosphokinase (U/l)	121 (89–183)	99 (74–189)	0.460	124 (81–181)	111 (74–189)	0.663
Prednisone						
Current use	6 (25)	7 (53.8)	0.096	5 (26.3)	8 (44.4)	0.298
Dose (mg/day)	6.3 (2.5–20.0)	5 (2.5–30.0)	0.945	10.0 (7.3)	9.1 (8.9)	0.847
Dose >10 mg/day	2 (8.3)	3 (23.1)	0.321	2 (10.5)	3 (16.7)	0.660
Immunosuppressive drugs	19 (79.2)	13 (100)	0.140	14 (73.7)	18 (100)	**0.046**
Mycophenolate mofetil	7 (29.2)	8 (61.5)	0.056	6 (31.5)	9 (50)	0.254
MTX	7 (29.2)	1 (7.7)	0.216	5 (26.3)	3 (16.7)	0.693
AZA	4 (16.7)	2 (15.4)	1.000	3 (15.7)	3 (16.7)	>0.999
LEF	3 (12.5)	0 0	0.538	2 (10.5)	1 (5.6)	>0.999
Ciclosporin	0	2 (15.4)	–	0	2 (11.1)	–
CYC	1 (4.2)	1 (7.7)	1.000	1 (5.3)	1 (5.6)	1.000
Rituximab	3 (12.5)	3 (23.1)	0.643	2 (10.5)	4 (22.2)	0.405

Results are expressed in mean (S.D.), median (interquartile range 25th–75th) and frequency (%). Bold text indicates significance. IMNM: immune-mediated necrotizing myopathies; Nab: neutralization antibodies; SAMs: systemic autoimmune myopathies; SC: seroconversion.

vaccine safety was demonstrated by the absence of severe or moderate adverse events related to vaccination with only mild and self-limiting side effects.

We observed that patients with SAMs had a moderate immune response to this vaccine and within the standards established by Food and Drugs Administration (FDA) and European Medicine Agency for Emergency Use Authorization of pandemic vaccines [30, 31]. In addition, the WHO recently approved the Sinovac-CoronaVac COVID-19 vaccine for emergency use [32]. However, after complete vaccination, the immunogenicity was lower compared with CTRL, but with SC rates comparable to the 64% reported for the pandemic influenza A H1N1 inactivated vaccine in a study of 1,600 autoimmune rheumatic disease patients [33]. Our findings with Sinovac-CoronaVac vaccine confirm and extends Furer *et al.*'s study [19] which assessed serum IgG antibody levels against SARS-CoV-2 proteins after the second dose of BNT162b2 mRNA COVID-19 vaccine and showed significantly reduced vaccine-induced immunogenicity in a small SAMs population ($n = 19$). We further demonstrated that NAb rates, now recognized as one of the major predictors of SARS-CoV-2 immune protection [34] were also moderate but lower than CTRL.

In contrast, after the first dose there was a negligible vaccine response (SC and NAb positivity) reinforcing the importance of the second dose for these patients. However, among patients who develop NAb, NAb activity was comparable for both groups after the first and second dose.

Further analysis of possible interference of clinical and laboratory parameters, comorbidities and type of SAMs in vaccine immunogenicity revealed that solely immunosuppressive drugs hampered the NAb positivity. This finding is in line with the reported reduced vaccine response in patients under mycophenolate mofetil therapy [17, 19, 20], rituximab [17–20], MTX [19, 20] and abatacept [19, 20] after different kinds of vaccines and their schedules [13–20]. Accordingly, in the present study, >80% of patients were under immunosuppressive drugs, especially mycophenolate mofetil in one third of

TABLE 5 Adverse events of Sinovac-CoronaVac vaccination in patients with systemic autoimmune myopathies and control group

	After vaccine first dose			After vaccine second dose		
	SAMs (*n* = 53)	CTRL (*n* = 106)	*P*-value	SAMs (*n* = 50)	CTRL (*n* = 106)	*P*-value
No symptoms	27 (50.9)	66 (62.3)	0.172	27 (54.0)	63 (59.4)	0.431
Local reactions[a]	11 (20.8)	18 (17.0)	0.561	11 (22.0)	19 (17.9)	0.579
Pain	9 (17.0)	15 (14.2)	0.638	11 (22.0)	17 (16.0)	0.390
Erythema	0	1 (0.9)	—	3 (6.0)	3 (2.8)	0.390
Swelling	0	4 (3.8)	—	4 (8.0)	6 (5.7)	0.728
Bruise	0	4 (3.8)	—	1 (2.0)	2 (1.9)	>0.999
Pruritus	2 (3.8)	1 (0.9)	0.258	2 (4.0)	6 (5.7)	>0.999
Induration	2 (3.8)	1 (0.9)	0.258	2 (4.0)	4 (3.8)	>0.999
Systemic reactions	23 (43.4)	34 (32.1)	0.161	16 (32.0)	31 (29.3)	0.775
Fever	2 (3.8)	0	—	0	3 (2.8)	—
Malaise	5 (9.4)	3 (2.8)	0.118	3 (6.0)	9 (8.5)	0.752
Somnolence	8 (15.1)	11 (10.4)	0.387	6 (12.0)	12 (11.3)	0.931
Lack of appetite	2 (3.8)	3 (2.8)	>0.999	1 (2.0)	5 (4.7)	0.664
Nausea	1 (1.9)	1 (0.9)	>0.999	1 (2.0)	10 (9.4)	0.104
Vomiting	0	0	—	0	1 (0.9)	—
Diarrhea	2 (3.8)	7 (6.6)	0.719	1 (2.0)	6 (5.7)	0.428
Abdominal pain	2 (3.8)	4 (3.8)	>0.999	2 (4.0)	5 (4.7)	>0.999
Vertigo	5 (9.4)	5 (4.7)	0.248	2 (4.0)	6 (5.7)	>0.999
Tremor	0	0	—	0	0	—
Headache	14 (26.4)	9 (8.5)	**0.002**	8 (16.0)	19 (17.9)	0.731
Fatigue	6 (11.3)	8 (7.5)	0.429	5 (10.0)	15 (14.1)	0.445
Sweating	2 (3.8)	3 (2.8)	>0.999	3 (6.0)	1 (0.9)	0.100
Myalgia	5 (9.4)	5 (4.7)	0.248	5 (10.0)	9 (8.5)	0.783
Muscle weakness	3 (5.7)	2 (1.9)	0.334	4 (8.0)	7 (6.6)	0.748
Arthralgia	4 (7.5)	6 (5.7)	0.732	5 (10.0)	8 (7.5)	0.627
Back pain	5 (9.4)	6 (5.7)	0.377	1 (2.0)	9 (8.5)	0.168
Cough	4 (7.5)	7 (6.6)	>0.999	3 (6.0)	7 (6.6)	>0.999
Sneezing	2 (3.8)	6 (5.7)	0.720	1 (2.0)	11 (10.4)	0.104
Coryza	1 (1.9)	10 (9.4)	0.101	3 (6.0)	8 (7.5)	>0.999
Stuffy nose	0 0	3 (2.8)	0.551	2 (4.0)	6 (5.7)	>0.999
Sore throat	3 (5.7)	5 (4.7)	>0.999	1 (2.0)	7 (6.6)	0.438
Shortness of breath	0	2 (1.9)	—	1 (2.0)	3 (2.8)	>0.999
Conjunctivitis	0	0	—	0	1 (0.9)	—
Pruritus	1 (1.9)	3 (2.8)	>0.999	1 (2.0)	5 (4.7)	0.664
Skin rash	1 (1.9)	2 (1.9)	>0.999	1 (2.0)	2 (1.9)	>0.999

Results are presented in frequency (%). Bold text indicates significance. [a]At the injection site. CTRL: control group; SAMs: systemic autoimmune myopathies.

patients, but also, at lower frequencies, MTX and rituximab. Although we could not show any specific drug effect due to the limited sample size, probably pooled analysis of these drugs was responsible for the interference in NAb positivity. In contrast to Furer *et al.* [19], that found a deleterious effect of glucocorticoids even at low dose [6.7 (6.3) mg/day of prednisone], we failed to show such interference with a very similar dose, also probably due to sample size.

Our patients had stable or low disease activity, according to inclusion criteria and IMACS core set measures at baseline and precluded any interpretation regarding the effect of disease activity in vaccine response, in spite of an association between mild elevated VAS of patient global activity and reduced frequency of NAb positivity. Therefore, further studies of SARS-CoV-2 vaccines with a large population of SAMs, including analysis of effect of individual immunosuppressive drugs, disease activity and different subtypes of SAMs will be necessary.

Patients with systemic autoimmune rheumatic diseases, including SAMs, may be at a higher risk for COVID-19 infection. Preliminary ACR guidelines recommended that patients with rheumatic and musculoskeletal diseases should be promptly vaccinated for COVID-19 [35]. Recent reports have also suggested that immunosuppressive drugs should be suspended for patients after COVID-19 vaccinations, particularly for

those under mycophenolate mofetil, MTX, CYC and rituximab to improve immunogenicity [36, 37]. Although our patients were in low disease activity, we choose not to withdraw medications due to the risk of reactivation and lack of definitive findings about each drug suspension at this specific population. Moreover, the current recommendations were not available during the study design.

There are limitations in the present study. First, inclusion of patients with different SAMs subtypes and from only one tertiary care centre, who may not represent the full spectrum of SAMs and could result in an overestimation of the disease activity or drug complications in the context of a more severe disease. Second, the sample size was not calculated because we used a convenience sample. Third, the FI-GMT and GMT values were not assessed for individual immunosuppressive drugs because of the small representation of each medication.

In conclusion, our data demonstrated that Sinovac-CoronaVac inactivated vaccine is safe and has a moderate short-term immunogenicity in inactive or low disease activity SAMs patients, although inferior compared with the CTRL. We further confirmed that immunosuppressive drugs have a deleterious effect on vaccine-induced antibody production, affecting in particular NAb positivity rates. These findings support the recommendation of SARS-CoV-2 vaccination for SAMs patients.

Acknowledgements

We thank the contribution of the Central Laboratory Division, Registry Division, Security Division, IT Division, Superintendence, Pharmacy Division and Vaccination Center (CRIE) for their technical support. We also thank the volunteers for handling the biological material and those responsible for the medical follow-up of all participants.

Funding: This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (#2015/03756–4 to N.E.A., S.G.P., C.A.S. and E.B., #2019/17272–0 to L.V.K.K., #2019/21211–6 to J.C.S.; #2019/11776-6 to S.K.S.); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq #305242/2019–9 to E.B., #304984/2020–5 to C.A.S., #303379/2018–9 to S.K.S.), B3 - Bolsa de Valores do Brasil to E.B., and Faculdade de Medicina da USP—SP to S.K.S. Instituto Butantan supplied the study product and had no other role in the trial.

Disclosure statement: The authors have declared no conflicts of interest.

Data availability statement

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study. Anonymised data are available on request from the corresponding author.

References

1 WHO. World alliance for patient safety: WHO draft guidelines for adverse event reporting and learning systems: from information to action 2021. https://www.who.int/covid-19/information (7 September 2021, date last accessed).

2 Barek MA, Aziz MA, Islam MS. Impact of age, sex, comorbidities and clinical symptoms on the severity of COVID-19 cases: meta-analysis with 55 studies and 10014 cases. Heliyon 2020;6:e05684.

3 Thakur B, Dubey P, Benitez J *et al.* A systematic review and meta-analysis of geographic differences in comorbidities and associated severity and mortality among individuals with COVID-19. Sci Rep 2021;11:8562.

4 Singh AK, Gillies CL, Singh R *et al.* Prevalence of comorbidities and their association with mortality in patients with COVID-19: a systematic review and meta-analysis. Diabetes Obes Metab 2020;22:1915–24.

5 Bower H, Frisell T, Di Giuseppe D *et al.* Impact of the COVID-19 pandemic on morbidity and mortality in patients with inflammatory joint diseases and in the general population: a nationwide Swedish cohort study. Ann Rheum Dis 2021;80:1086–93.

6 Strangfeld A, Schäfer M, Gianfrancesco MA *et al.* Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician-reported registry. Ann Rheum Dis 2021;80:930–42.

7 Dalakas MC. Inflammatory muscle diseases. N Engl J Med 2015;373:393–4.

8 Lundberg IE, Tjärnlund A, Bottai M *et al.* 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Arthritis Rheumatol 2017;69:2271–82.

9 Pinal-Fernandez I, Casal-Dominguez M, Mammen AL. Immune-mediated necrotizing myopathy. Curr Rheumatol Rep 2018;20:21.

10 Gupta L, Lilleker JB, Agarwal V, Chinoy H, Aggarwal R. COVID-19 and myositis - unique challenges for patients. Rheumatology 2021;60:907–10.

11 Marie I, Ménard JF, Hachulla E *et al.* Infectious complications in polymyositis and dermatomyositis: a series of 279 patients. Semin Arthritis Rheum 2011;41:48–60.

12 De Souza FHC, de Araújo DB, Vilela VS *et al.* Guidelines of the Brazilian Society of Rheumatology for the treatment of systemic autoimmune myopathies. Adv Rheumatol 2019;59:6.

13 Simon D, Tascilar K, Fagni F *et al.* SARS-CoV-2 vaccination responses in untreated, conventionally treated and anticytokine-treated patients with immune-mediated inflammatory diseases. Ann Rheum Dis 2021;80:1312–6.

14 Geisen UM, Berner DK, Tran F *et al.* Immunogenicity and safety of anti-SARS-CoV-2 mRNA vaccines in patients with chronic inflammatory conditions and immunosuppressive therapy in a monocentric cohort. Ann Rheum Dis 2021;80:1306–11.

15 Braun-Moscovici Y, Kaplan M, Braun M *et al.* Disease activity and humoral response in patients with inflammatory rheumatic diseases after two doses of the Pfizer mRNA vaccine against SARS-CoV-2. Ann Rheum Dis 2021;80:1317–21.

16 Cherian S, Paul A, Ahmed S *et al.* Safety of the ChAdOx1 nCoV-19 and the BBV152 vaccines in 724 patients with rheumatic diseases: a post-vaccination cross-sectional survey. Rheumatol Int 2021;41:1441– 5.

17 Boyarsky BJ, Ruddy JA, Connolly CM *et al.* Antibody response to a single dose of SARS-CoV-2 mRNA vaccine in patients with rheumatic and musculoskeletal diseases. Ann Rheum Dis 2021;80:1098–9.

18 Spiera R, Jinich S, Jannat-Khah D. Rituximab, but not other antirheumatic therapies, is associated with impaired serological response to SARS- CoV-2 vaccination in patients with rheumatic diseases. Ann Rheum Dis 2021;80:1357–9.

19 Furer V, Eviatar T, Zisman D *et al.* Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in adult patients with autoimmune inflammatory rheumatic diseases and in the general population: a multicentre study. Ann Rheum Dis 2021;80:1330–8.

20 Medeiros-Ribeiro AC, Aikawa NE, Saad CGS *et al.* Immunogenicity and safety of an inactivated virus vaccine against SARS-CoV-2 in patients with autoimmune rheumatic diseases. Nat Med 2021;27:1744–51.

21 Behrens Pinto GL, Carboni RCS, de Souza FHC, Shinjo SK. A prospective cross-sectional study of serum IL-17A in antisynthetase syndrome. Clin Rheumatol 2020;39: 2763–1.

22 Rider LG, Giannini EH, Harris-Love M *et al.* Defining clinical improvement in adult and juvenile myositis. J Rheumatol 2003;30:603–17.

23 Harris-Love MO, Shrader JA, Koziol D *et al.* Distribution and severity of weakness among patients with polymyositis, dermatomyositis and juvenile dermatomyositis. Rheumatology 2009;48:134–9.

24 Miller FW, Rider LG, Chung YL *et al.* Proposed preliminary core set measures for disease outcome assessment in adult and juvenile idiopathic inflammatory myopathies. Rheumatology 2001;40:1262–73.

25 Taylor SC, Hurst B, Charlton CL *et al.* A new SARS-CoV-2 dual-purpose serology test: highly accurate infection tracing and neutralizing antibody response detection. J Clin Microbiol 2021;59:e02438–20.

26 Corman VM, Landt O, Kaiser M *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25:2000045.

27 Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering how biological sex impacts immune responses and COVID-19 outcomes. Nat Rev Immunol 2020;20:442–7.

28 Fathi A, Addo MM, Dahlke C. Sex differences in immunity: implications for the development of novel vaccines against emerging pathogens. Front Immunol 2020;11:601170.

29 Shinjo SK, De Moraes JC, Levy-Neto M *et al.* Pandemic unadjuvanted influenza A (H1N1) vaccine in dermatomyositis and polymyositis: immunogenicity independent of therapy and no harmful effect in disease. Vaccine 2012;31:202–6.

30 European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP). Guideline on risk management systems for medicinal products for human use. 2018. https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-clinical-evaluation-vaccines-revision-1_en.pdf (7 September 2021, date last accessed).

31 U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research. Guidance for Industry: Clinical data needed to support the licensure of pandemic influenza vaccines. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research. 2007. https://www.fda.gov/files/vaccines,%20blood%20&%20biologics/published/Guidance-for-Industry–Clinical-Data-Needed-to-Support-the-Licensure-of-Pandemic-Influenza-Vaccines.pdf (7 September 2021, date last accessed).

32 WHO. WHO validates Sinovac COVID-19 vaccine for emergency use and issues interim policy recommendations 2021. https://www.who.int/news/item/01-06-2021-who-validates-sinovac-covid-19-vaccine-for-emergency-use-and-issues-interim-policy-recommendations (7 September 2021, date last accessed).

33 Saad CGS, Borba EF, Aikawa NE *et al.* Immunogenicity and safety of the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune rheumatic diseases. Ann Rheum Dis 2011;70:1068–73.

34 Khoury DS, Cromer D, Reynaldi A *et al.* Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med 2021;27:1205–11.

35 Curtis JR, Johnson SR, Anthony DD *et al.* American College of Rheumatology Guidance for COVID-19 vaccination in patients with rheumatic and musculoskeletal diseases: version 1. Arthritis Rheumatol 2021;73:1093–107.

36 Moutsopoulos HM. A recommended paradigm for vaccination of rheumatic disease patients with the SARS-CoV-2 vaccine. J Autoimmun 2021;121:102649.

37 Arnold J, Winthrop K, Emery P. COVID-19 vaccination and antirheumatic therapy. Rheumatology 2021;60: 3496–502.

CoronaVac

O que a ciência comprova

4.9. CoronaVac traz níveis elevados de proteção para pessoas com HIV, indicam estudos do Brasil e da China

Dois estudos científicos publicados por pesquisadores do Brasil e da China evidenciam que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, é segura e capaz de gerar níveis elevados de proteção contra o SARS-CoV-2 em pessoas infectadas pelo vírus HIV, causador da AIDS.

O trabalho "Safety and Immunogenicity of CoronaVac in People Living with HIV", realizado por pesquisadores do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo e publicado na plataforma de preprints SSRN, avaliou a segurança e imunogenicidade da CoronaVac em 215 pessoas que vivem com HIV, na comparação com 296 pessoas sem imunossupressão conhecida. Todos os participantes receberam duas doses de CoronaVac com um intervalo de 28 dias.

Quatro semanas após a segunda dose da vacina, a porcentagem de participantes com positividade para anticorpos neutralizantes SC e NAb foi alta tanto para o grupo com HIV quanto no grupo controle. Nenhuma reação adversa séria foi relatada durante o estudo, seja entre pessoas com HIV ou nos participantes não imunossuprimidos.

No entanto, os pesquisadores encontraram diferenças nos parâmetros de imunogenicidade entre as pessoas com HIV. Os linfócitos T CD4 (células CD4) ajudam a coordenar a resposta imune, estimulando outras células imunes como os linfócitos B (células B) e T CD8 (células CD8) a combater a infecção. O vírus HIV enfraquece o sistema imunológico, destruindo as células CD4. Decorridos 69 dias da primeira dose da CoronaVac, os participantes com contagem de células T CD4 menor que 500 células/mm^3 tinham imunogenicidade mais baixa contra o vírus SARS-CoV-2 quando comparados aos membros do mesmo grupo com contagem maior ou igual a 500 células por mm^3.

A partir dessa análise, os pesquisadores concluíram que as pessoas com HIV e contagem maior ou igual a 500 células T CD4 por mm^3 tinham 2,26 vezes mais chances de apresentar positividade na atividade dos anticorpos neutralizantes

quando comparadas aos com contagem de células T CD4 por mm^3 menor que 500. Em relação aos participantes do grupo controle, esse indicador era 3,21 vezes maior.

"Nossos resultados mostraram que a CoronaVac tem imunogenicidade robusta em pessoas vivendo com HIV após um regime de duas doses, mas as respostas de anticorpos nesta população são um pouco mais baixas do que em indivíduos não imunossuprimidos", afirmam os autores. "Estratégias devem ser desenvolvidas para melhorar a imunogenicidade induzida por vacina entre as pessoas vivendo com HIV, especialmente no subgrupo com baixas contagens de células T CD4. Uma abordagem possível é usar uma dose de vacina de reforço ou mesmo administrar títulos de antígeno mais altos por dose de vacina", concluem eles.

Outro estudo realizado por pesquisadores chineses e publicado na plataforma SSRN também trouxe evidências de que a CoronaVac é segura para pessoas vivendo com o vírus HIV, e que as pessoas deste grupo, quando totalmente imunizadas no esquema de duas doses da vacina do Butantan, podem alcançar níveis elevados de proteção contra o SARS-CoV-2, similares aos observados nos indivíduos HIV-negativos.

A Covid-19 e o HIV

Um relatório publicado em julho de 2021 pelo Programa Conjunto das Nações Unidas sobre HIV/AIDS (UNAIDS) analisou mais de 168 mil pessoas hospitalizadas com Covid-19 em todo o mundo e concluiu que a incidência da forma mais grave da doença e o número de mortes intra-hospitalares eram maiores em pessoas que vivem com HIV, independentemente de idade, sexo e comorbidades. Estima-se que mais de 38 milhões de pessoas vivam com HIV em todo o mundo, sendo 1 milhão delas no Brasil.

Publicados em: 12/10/2021 e 22/09/2021

Artigo

Safety and immunogenicity of CoronaVac in people living with HIV

Lucas Chaves Netto[1], Karim Yaqub Ibrahim[1], Camila de Melo Picone[1], Ana Paula Pereira da Silva Alves[1], Eliane Vieira Aniceto, Mariana Rodrigues Santiago[1], Patrícia da Silva Spindola Parmejani[1], Nadia E Aikawa[2], Ana C Medeiros-Ribeiro[2], Sandra G Pasoto[2], Emily F N Yuki[2], Carla G S Saad[2], Tatiana Pedrosa[2], Amanda Nazareth Lara[1], Carina Ceneviva[3], Eloisa Bonfa[2], Esper Georges Kallas[1]*, Vivian I. Avelino-Silva[1]*

1. Department of Infectious and Parasitic Diseases, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil
2. Rheumatology Division, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil
3. Department of Pathology, Central Laboratory Division, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil Address: Av. Dr. Eneas Carvalho de Aguiar, 155 - 2º Andar - Cerqueira Cesar – CEP 01246-100 - Sao Paulo/SP – Brazil

*Equal contribution

Keywords: SARS-CoV-2; COVID-19; vaccine; HIV; immunogenicity; neutralizing antibodies; CoronaVac

Word count: 3,049

Abstract:

Background: People living with HIV (PLWH) may have a poor or delayed response to vaccines, mainly when CD4+ T cell counts are low. There are limited data concerning the safety and immunogenicity of COVID-19 vaccines in PLWH.

Methods: This prospective controlled study evaluated the safety and immunogenicity of the SARS-CoV-2 inactivated vaccine CoronaVac in PLWH compared with controls with no known immunosuppression. Immunogenicity was assessed with SARS-CoV-2 IgG seroconversion (SC), neutralizing antibodies (NAb) activity, and factor increase in IgG geometric mean titers (FI-GMT). We also investigated if levels of CD4+ T cell counts (< or ≥500 cells/mm^3) were associated with CoronaVac immunogenicity.

Findings: 511 participants (215 PLWH and 296 controls) were eligible for the immunogenicity analysis. At vaccine completion (D69), although the percentage of participants with SC and NAb positivity was high for both PLWH and controls, it was somewhat lower in PLWH. CD4+ T cell was identified as a relevant factor for immunogenicity, with lower SC and NAb positivity in PLWH with CD4+ counts <500 cells/mm^3 compared to those with ≥500 cells/mm^3. In a

multivariable logistic regression model for NAb positivity after a complete two-dose regimen adjusted for age and sex, compared with PLWH with a CD4+ T cell count <500/mm^3, those with CD4+ counts ≥500/mm3 had 2·26 times the odds of having positivity in NAb activity (95% CI 1·18-4·32; p=0·014), whereas controls had 3·21 times the odds of this outcome. No serious adverse reactions were reported during the study.

Interpretation: Immunogenicity following CoronaVac in PLWH seems robust but reduced compared with controls; PLWH with CD4+ counts <500/mm^3 are at increased risk for a blunted antibody response following vaccination.

Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); and B3 - Bolsa de Valores do Brasil.

Research in context:

Evidence before this study: Several studies have shown that people living with HIV (PLWH) may have a poor or delayed response to vaccines or even a reduced duration of immunogenicity following vaccination. So far, scarce data concerning safety and immunogenicity of COVID-19 vaccines in PLWH is available.

Added value of this study: This is the first controlled study addressing safety and immunogenicity of the SARS-CoV-2 inactivated vaccine CoronaVac in PLWH compared with controls with no known immunosuppression. At four weeks after the second vaccine dose, the percentage of participants with seroconversion and neutralizing antibodies positivity was high for both PLWH and controls. However, the study found significantly lower immunogenicity among PLWH compared to non-immunosuppressed participants. Moreover, PLWH with CD4+ T cell counts <500 cells/mm^3 had lower SARS-CoV-2 immunogenicity compared to PLWH with CD4+ T cell counts ≥500 cells/mm^3 and

Implications of all the available evidence: Strategies to improve vaccine-induced immunogenicity may be needed for PLWH. Data on clinical efficacy and real-life effectiveness studies are still lacking for this population.

Introduction:

Several vaccines have been implemented in clinical practice to prevent severe COVID-19 cases and related deaths. Brazil has been severely hit by the pandemic, with one of the highest rates of reported cases and deaths globally.[1] Up to September 2021, four vaccines have been implemented in Brazil; the ChAdOx1 by AstraZeneca and the CoronaVac by Sinovac and Butantan Institute have been more frequently used, followed by a more recent introduction of the single-dose Ad26.COV2.S by Janssen and the BNT162b2 by Pfizer and BioNTech . Compared to other COVID-19 vaccines, CoronaVac has logistical advantages in storage (requiring refrigeration only) and manufacturing technology. Mass vaccinations campaigns have already taken place in Turkey, Brazil, Chile, and Indonesia, with approval for emergency use in more than 20 low and middle-income countries.[2,3]

Several risk factors have been associated with poor outcomes among COVID-19 cases, including pulmonary, cardiac, and chronic renal conditions; older age; obesity; and immunosuppression such as solid organ transplants, recent chemotherapy, hematopoietic diseases, and HIV infection. Although large cohorts from United States, United Kingdom, and South Africa showed an increased risk of COVID-19-associated death among PLWH compared to HIV-uninfected individuals after adjustment for covariates[4], some observational and epidemiological data suggested no more significant risk, especially among PLWH with well-controlled HIV infection.[5] However, several studies demonstrate that PLWH may have a poor or delayed response to vaccines or even a reduced duration of immunogenicity following vaccination against *Pneumococcus sp*, Influenza, Hepatitis A and B[6], and Yellow Fever.[7]

So far, scarce safety data concerning PLWH vaccinated with COVID-19 vaccines is available, with only 0·6% and 0·5% representation of PLWH in clinical trials with the mRNA-1273 and BNT162b2 vaccines, respectively.[8,9] In a small cohort of 12 PLWH vaccinated with the mRNA vaccine, lower immunogenicity was observed among those with CD4+ T cell counts <200/mm^3.[9] There is also limited data regarding the use of ChAdOx1 in this population from a South African cohort (102 PLWH vs. 56 controls) and a subgroup analysis of a phase 2/3 study in England (54 PLWH), with no significant differences in immunogenicity.[10] There are, however, no data on the safety and immunogenicity of inactivated COVID-19 vaccines in PLWH to date.

This cohort study evaluated the safety and immunogenicity of the SARS-CoV-2 inactivated vaccine CoronaVac in PLWH compared with controls with no known immunosuppression.

Methods

Study design and population

In this prospective cohort nested within a large phase 4 vaccination protocol (clinicaltrials.gov #NCT04754698), PLWH aged >18 years regularly followed at the HIV/AIDS outpatient clinic at the University of São Paulo were invited to participate. We included adults with no known immunosuppression who received CoronaVac as controls. We excluded potential participants with a history of anaphylactic reaction to the vaccine components; acute febrile illness at vaccination; current hospitalization; a history of Guillain-Barre syndrome or demyelinating disease; previous vaccination with any SARS-CoV-2 vaccine; a history of vaccination with a live virus vaccine up to four weeks before enrolment, or an inactivated vaccine up to two weeks before enrolment; and a history of any blood product transfusion up to 6 months before enrolment. Participants with well-controlled comorbidities were included, but those reporting other types of immunosuppression or COVID-19 symptoms at the time of the first vaccine dose were excluded. Participants with positive results in baseline assessment of SARS-CoV-2 IgG or neutralizing antibodies (NAb) were also excluded from the analysis.

Study procedures

We collected demographic and clinical characteristics of study participants at baseline, and laboratory variables including last CD4+ T cell count and HIV viral load were extracted from medical charts. CoronaVac was administered in a twice-dose regimen 28 days apart, according to the manufacturer's recommendations.[11] CoronaVac (Sinovac Life Sciences, Beijing, China, batch #20200412) contains a β-propiolactone inactivated SARS-CoV-2 derived from the CN02 strain of SARS-CoV-2 grown in African green monkey kidney cells - Vero 25 cells with aluminum hydroxide as an adjuvant. Single-use CoronaVac syringes containing 0·5 mL were administered intramuscularly in the deltoid area. Participants underwent blood collections immediately before each vaccine administration and four weeks after the second dose (D69). Serum samples were stored at -70˚C. In case of incident COVID-19 during the study period, the second vaccination was delayed by four weeks.

Immunogenicity evaluation

The immunogenicity evaluation comprised two serologic tests: a chemiluminescent immunoassay that measured IgG antibodies targeting S1 and S2 proteins in receptor binding domain (Indirect ELISA, LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy), measured in AU/mL (Arbitrary Units) and a virus NAb detection assay SARS-CoV-2 sVNT Kit (GenScript, Piscataway, NJ, USA). Seroconversion (SC) was defined as a positive (≥15·0 AU/mL) serology for the IgG test. We also calculated IgG geometric mean titers (GMT) and 95% confidence intervals at all time points and the factor increase in GMT (FI-GMT) as the ratio of the GMT after vaccination to the GMT before vaccination. NAb activity was reported as percentages and categorized as positive when ≥30% as suggested by the manufacturer.[12] Immunogenicity tests were performed in samples collected at baseline (D0), immediately before the second vaccine shot (D28, intermediary assessment), and six weeks after the second vaccine dose (D69, final assessment).

Safety evaluation

The vaccine's local and systemic side effects were monitored using a standardized form and clinical evaluations at each study visit. Participants completed the standardized forms with solicited adverse reactions after each vaccine dose. Solicited local adverse reactions included pain, erythema, swelling, bruise, pruritus, and induration at the vaccine injection site. Systemic reactions included fever, malaise, somnolence, lack of appetite, sweating, nausea, vomit, diarrhea, abdominal pain, vertigo, tremor, headache, fatigue, myalgia, muscle weakness, arthralgia, back pain, cough, sneezing, coryza, runny nose, sore throat, shortness of breath, conjunctivitis, pruritus and skin rash.

Moderate and severe adverse events have been recorded from D0-D69 and classified as vaccine-related and unrelated. Participants with COVID-19 symptoms during the study period underwent a SARS-CoV-2 reverse transcriptase–polymerase-chain-reaction (RT-PCR) test in a nasal swab sample.

Statistical analysis:

We present the characteristics of study participants using descriptive statistics. Comparisons between PLWH and non-immunosuppressed controls were made using Mann-Whitney-Wilcoxon rank-sum tests for numeric variables and chi-squared or Fisher's exact tests for categorical variables. We generated categorical variables for age (<40; 40-49; 50-49; ≥60 years old), and $CD4^+$ T cell counts (<500; ≥500). A multivariable logistic regression model was used to assess the impact of HIV infection, and CD4+ T cell counts on the positivity of SARS-CoV-2 anti-S1/S2 IgG and NAb test following vaccination, adjusted for age and sex. We used the statistical software Stata 15·1 (StataCorp College

Station, TX: StataCorp LP) in all analyzes, with a two-tailed significance level of 0·05.

Ethical aspects

The national and local ethics committees approved the study. Each participant provided written informed consent before enrolment. Participant identifiable data remained confidential throughout the study.

The study sponsors had no role in study design, data collection, analysis, interpretation of data, writing of the report, or in the decision to submit the paper for publication

Results

Between February and March 2021, 776 consecutive participants were recruited, of whom 282 were PLWH and 494 non-immunosuppressed controls. Two participants from the control group were excluded after drop-out following the first vaccine dose. Additional 244 (31%) individuals were excluded from this analysis due to a positive IgG or NAb test at baseline (53 PLWH [19%] and 191 controls [39%]), and 19 individuals were excluded due to missing baseline results of IgG or NAb tests. The remaining 511 individuals comprised the study sample for the immunogenicity analysis (215 PLWH and 296 non-immunosuppressed controls). For the safety analysis, 465 participants completed the forms. A flowchart describing study participants is presented in Supplement Figure 1.

Demographic and clinical characteristics of study participants are presented in Table 1. Female participants comprised 85 (40%) of the PLWH and 187 (63%) of the non-immunosuppressed participants (p<0·001). PLWH were older than controls, with a median 54 years old (interquartile range [IQR] 45-60) and 48 years old (IQR 37-58), respectively (p<0·001).

The frequency of comorbidities was similar between PLWH and controls, except for a higher frequency of dyslipidemia (17% *vs*. 5%; p<0·001) and chronic kidney disease (2% *vs*. 0%; p 0·013) among PLWH.

We obtained CD4+ T cell counts of all 215 PLWH, with a median of 22 months from the last CD4+ T cell count measurement and study enrolment (IQR 11-33). CD4+ T cell counts were <500 cells/mm^3 for 64 (30%) participants and ≥500 cells/mm^3 for the remaining 151 (70%). Overall, 191 (89%) PLWH had undetectable (<50 copies/mL) viral load in at least three measurements before inclusion and were considered with viral suppression. The median time between the last HIV viral load assessment and study enrolment was two months (IQR 1-3).

SARS-CoV-2 vaccine immunogenicity: effect of HIV infection

Table 2 describes results of the immunogenicity assessment. In unadjusted analysis at vaccine completion (D69), the frequency of positive SARS-CoV-2 IgG SC and NAb positivity was high for both PLWH and non-immunosuppressed controls; it was significantly lower in PLWH (SC 91 vs. 97%, p<0·005; NAb positivity 70·7 vs. 84%, p<0·001). The FI-GMT and NAb activity were moderate and lower in PLWH compared to non-immunosuppressed controls [median FI-GMT 22·5 (IQR 10·9 – 41·1) vs. 31·8 (IQR 15 – 53·1), p<0·001; median NAb activity 46·1 (26·9 – 69·7) vs. 60·7 (39·8 – 79·9), p<0·001]. Of note, at the day of the second dose (D28), PLWH had lower percentages of SARS-CoV-2 IgG SC (19 vs. 39%, p<0·001), NAb positivity (19 vs. 39%, p<0·001), and lower levels of FI-GMT (2·3 vs. 4·6, p<0·001) and NAb activity (0 vs. 23.7%, p<0·001) compared to non-immunosuppressed controls.

SARS-CoV-2 vaccine immunogenicity: effect of CD4+ T cell counts among PLWH

In the final assessment (D69), PLWH with CD4+ T cell counts <500 cells/mm^3 had a lower immunogenicity compared to those with CD4+ T cell counts ≥500 cells/mm^3 [SC 82 vs. 94%, p=0·008; NAb positivity 59 vs. 76, p=0·001; median NAb activity: 41·6 vs. 49·9%, p=0·030]. At D28, PLWH with CD4+ T cell counts < or ≥500/mm^3 had comparable immunogenicity parameters (p>0·05) except for the NAb activity (0 vs. 23·7%, p=0·002; Table 2). Figure 1 shows the final SARS-CoV-2 NAb activity among PLWH with CD4+<500 cells/mm^3, CD4+≥500 cells/mm^3 and HIV-uninfected participants; the median final NAb activity was 41·6 % (IQR 20·8 – 64·6) among PLWH with <500 cells/mm^3; 49·9 % (IQR 30·6 – 73·1) for PLWH with ≥500 cells/mm^3; and 60·8 % (IQR 39·8 – 79·9) among HIV-uninfected participants.

Multivariable analysis for SARS-CoV-2 vaccine immunogenicity

Given the baseline differences between groups regarding sex and age distributions, we performed a multivariable logistic regression including HIV status and CD4+ T cell counts (< or ≥500/mm3), with age categories and sex as independent variables, and positivity in NAb at the final study assessment (D69) as the outcome.

The model showed that, compared with PLWH with a CD4+ T cell count <500/mm^3, those with CD4+ counts ≥500/mm3 had 2·26 times the odds of having a positive NAb after complete vaccination (D69) (95% CI 1·18-4·32; p=0·014), whereas HIV-uninfected individuals had 3·21 times the odds of this

outcome (95% CI 1·72-6·00; p<0·001). Female sex and age categories were not significantly associated with the odds of having a positive NAb (Table 3).

Vaccine safety

Information regarding adverse vaccine reactions was available for 189 PLWH and 296 non-immunosuppressed participants. Adverse events are detailed in Supplement Table 1, and the most frequently reported symptoms are presented in Figure 2. Most participants were asymptomatic after vaccination with the first (61%) and the second (68%) vaccine dose. Only mild adverse events were reported during the study. PLWH and non-immunosuppressed participants had no statistically significant differences in the occurrence of vaccine adverse events after the first dose, except for any local reactions (12% vs. 21% respectively; p=0·026) and sweating (5% vs. 1% respectively; p=0·005).
After the second shot, we found a higher frequency of adverse reactions among non-immunosuppressed participants, including nausea (2% *vs*. 6%; p=0·013), myalgia (4% *vs*. 8%; p=0·048), arthralgia (3% *vs*. 8%; p=0·048), shortness of breath (0 *vs*. 3%; p=0·016), and pruritus (0% *vs*. 3%; p=0·016) compared to PLWH.

Supplement Figure 1: Selection of study participants

Table 1: Demographic and clinical characteristics of participants eligible for immunogenicity analysis

	PLWH N=215	Non-immunosuppressed controls N=296	p-value
Age category (%)			
<40 years old	34 (16)	88 (30)	
40 – 49 years old	45 (21)	75 (25)	
50 – 59 years old	69 (23)	69 (23)	
>60 years old	64 (22)	64 (22)	
Median Age (IQR)	54 (45-60)	48 (37 – 58)	<0·001
Female sex, n (%)	85 (40)	187 (63)	<0·001
CD4+ category, cells/mm³, n (%)			
CD4+ < 200	9 (4)	-	-
CD4+ 200 – 349	24 (11)	-	-
CD4+ 350 – 499	31 (14)	-	-
CD4+ ≥ 500	151 (70)	-	-
Median CD4+ count (IQR)	655 (458 – 900)	-	-
Viral suppression, n (%)	191 (89)	-	-
Median weeks between last CD4+ count and inclusion (IQR)	21 (10 – 33)	-	-
Comorbidities, n (%)			
Smoking	28 (13)	33 (11)	0·305
Hypertension	52 (24)	71 (24)	0·520
Diabetes	27 (13)	37 (13)	0·544
Cardiopathy	5 (2)	4 (1)	0·310
Dyslipidemia	37 (17)	15 (5)	<0·001
COPD	0	3 (1)	0·194
Asthma	5 (2)	10 (3)	0·338
Chronic kidney disease	5 (2)	0	0·013
Chronic liver disease	4 (2)	1 (<1)	0·103
Neoplasia	2 (1)	0	0·177
Previous stroke	5 (2)	0	0·013
Active tuberculosis	2 (1)	0	0·177

COPD: chronic obstructive pulmonary diseases

Table 2: Immunogenicity after one dose (D28) and two doses (D69) for PLWH, according to CD4+ counts category, and non-immunosuppressed controls

	HIV-uninfected N = 296	PLWH N = 215	*P*-value comparing PLWH and controls	PLWH CD4+ < 500 N = 64	PLWH CD4+ ≥ 500 N = 151	*P*-value comparing high and low CD4+
D69						
IgG levels (AU/mL)	75·2 (50·3 – 112)	48·7 (26·5 – 88·2)	<0·001	42·0 (22·9 – 68·9)	53·3 (30·2 – 92·4)	0·053
Seroconversion	265 / 274 (97%)	185 / 204 (91%)	0·005	51 / 62 (82%)	134/ 142 (94%)	0·008
FI-GMT	31·8 (16 – 53·1)	22·5 (10·9 – 41·1)	<0·001	19·3 (7·6 – 33·5)	23·0 (11 – 45)	0·120
NAb positivity	229 / 274 (84%)	143 / 202 (71%)	0·001	36 / 61 (59%)	107 / 141 (76%)	0·013
Percent NAb activity	60·7 (39·8 – 79·9)	46·1 (26·9 – 69·7)	<0·001	41·6 (20·8 – 64·6)	49·9 (30·6 – 73·1)	0·030
D28						
IgG levels (AU/mL)	10·4 (4·7 – 30·5)	5·1 (0 – 11·3)	<0·001	5·1 (0 – 7·9)	5·1 (0 – 12·3)	0·448
Seroconversion	114 / 295 (39%)	41 / 214 (19%)	<0·001	10 / 64 (15%)	31 / 150 (20%)	0·255
FI-GMT	4·6 (2·3 – 10·3)	2·3 (1·0 – 5·2)	<0·001	2·2 (1 – 3·8)	2·4 (1 – 6)	0·337
NAb positivity	112 / 289 (39%)	40 / 211 (19%)	<0·001	7 / 64 (11%)	33/147 (22%)	0·035
Percent NAb activity (%)	23·7 (0 – 39·6)	0 (0 – 27·3)	<0·001	0 (0 – 0)	23·7 (0 – 39·6)	0·002

Numeric variables are presented as medians and interquartile ranges; categorical variables are presented as frequencies and percentages; AU: arbitrary units; SC: seroconversion (positive IgG, ≥15AU/mL); NAb: Neutralizing antibody test (positive when ≥ 30%); FI-GMT: factor of increase – geometric mean titter

Figure 1: SARS-CoV-2 percentage neutralizing antibodies activity among persons living with HIV with CD4<500, CD4≥500, and non-immunosuppressed participants. Dots represent results from individual vaccines; whiskers indicate 25th, 50^{th,} and 75th percentiles.

Table 3: Multivariable logistic regression model for neutralizing antibody positivity after vaccination with a two-dose regimen of inactivated SARS-CoV-2 vaccine, according to HIV status and CD4+ T cell counts

	OR	95% CI	p-value
PLWH, CD4+<500 mm^3	Reference (1·00)	-	-
PLWH, CD4+≥500 mm^3	2·26	1·17 – 4·32	0·014
Non-immunosuppressed participants	3·21	1·72 – 5·99	<0·001
Female sex	1·17	0·73 – 1·85	0·510
Age category			
<40 years old	Reference (1·00)	-	-
40 – 49 years old	1·06	0·51 – 2·18	0·871
50 – 59 years old	0·77	0·40 – 1·56	0·512
>60 years old	0·55	0·28 – 1·07	0·082

PLWH: People living with HIV

Figure 2: Local (panel A) and systemic (panel B) adverse events after vaccination, according to vaccine dose and HIV infection status

Discussion

Here we present the findings of the first controlled study addressing the safety and immunogenicity of an inactivated vaccine against SARS-CoV-2 among PLWH compared with non-immunosuppressed controls. No serious adverse reactions were reported during the study, either among PLWH or non-immunosuppressed participants. We found a few statistically significant differences with a higher occurrence of adverse reactions in the control group compared to PLWH. At four weeks after the second vaccine dose, the percentage of participants with SC and NAb positivity was high for both PLWH and controls. However, we found statistically significant differences in the immunogenicity parameters comparing PLWH and non-immunosuppressed participants in unadjusted analysis both after the first dose and after the second vaccine. In addition, at D69, PLWH with CD4+ T cell counts <500 cells/mm^3 had lower SARS-CoV-2 immunogenicity compared to PLWH with CD4+ T cell counts ≥500 cells/mm^3.

We observed a few differences between PLWH and non-immunosuppressed participants in baseline demographics and clinical characteristics. Female sex was more frequent among non-immunosuppressed controls, and PLWH were somewhat older. Both factors have been adjusted for in the multivariable model. Regarding comorbidities, the only significant differences were a higher frequency of dyslipidemia (17% *vs*. 5%) and chronic kidney diseases (2% *vs*. 0%) among PLWH. The higher occurrence of chronic non-communicable diseases in PLWH is a documented phenomenon.[13] Due to a low overall frequency, we did not include these variables as covariates in the multivariable model addressing immunogenicity. Our multivariable logistic regression model for NAb positivity at D69 adjusted for age and sex showed that non-immunosuppressed participants and PLWH with CD4+ T cell count ≥500/mm^3 had significantly higher odds of having a positive NAb compared to PLWH with CD4+ T cell count <500/mm^3.

Our results are consistent with previous knowledge on the immunogenicity elicited by vaccines among PLWH and patients with lower CD4+ T cell counts.[6] HIV infection is known to impair the immune system beyond the decrease of CD4+ T cell counts,[14] impacting various immunologic pathways resulting in immune activation, impaired humoral and cellular responses, and clinical

outcomes including a decreased immunogenicity to several vaccines. Studies have shown that vaccines such as the live attenuated Yellow Fever vaccine, inactivated tetravalent influenza and hepatitis A/B vaccines, pneumococcal (both polysaccharide [PPSV 23] and conjugated formulations [PCV10, PCV13]) and conjugated *Haemophilus influenzae* type B elicit a less robust immune response in PLWH compared with HIV-uninfected individuals regardless of antiretroviral treatment and CD4+ T cell counts. [7,15,16] Moreover, the vaccine-induced immune response seems to be particularly impaired in situations of advanced or uncontrolled HIV infection, with low CD4+ T cells (<200/mm^3) and detectable HIV viral load.[6] Studies also suggest that the vaccine-induced immunogenicity may wane more rapidly for this group of patients.[17]

Recent studies on the immunogenicity of COVID-19 vaccines in immunosuppressed patients suggest that the antibody response may be impaired in these populations. Medeiros-Ribeiro et al. published a phase IV controlled study assessing immunogenicity following CoronaVac among patients with autoimmune rheumatologic diseases and found a NAb positivity of 56% compared to 79% among controls.[18] Additional studies addressing other COVID-19 vaccines such as the mRNA Pfizer BioNTech also found a reduced antibody response in immunosuppressed patients such as chronic corticosteroid users,[19] patients under immunosuppressive drugs,[20] and solid organ transplant recipients.[21,22]

Our study had a few limitations. As seen in any observational study, groups were subject to imbalances in demographic and clinical characteristics. The older age and lower frequency of female sex among PLWH could partially explain the lower immune response to the inactivated SARS-CoV-2 vaccine, as older age has been associated with lower vaccine immunogenicity[23] and female sex was associated with higher vaccine immunogenicity and reactogenicity.[24] This imbalance could also partially explain the higher frequency of adverse reactions in the non-immunosuppressed group. We fit a multivariable logistic regression model including sex and age categories to adjust for these imbalances. Interestingly, in this model, sex and age categories had no statistically significant impact on final NAb positivity, whereas HIV status and CD4+ T cell count categories remained associated with final NAb positivity. Another limitation was the use of broad CD4+ T cell count categories due to the low number of participants with CD4+ T cell count<350/mm^3. As such, we were unable to explore the effect of lower levels of CD4+ T cells on vaccine immunogenicity. Other potential problems include the lack of recent CD4+ T cell count measurements for some PLWH, with a median of 22 months between the last

assessment and study enrolment. The current Brazilian HIV treatment guidelines recommend avoiding CD4+ T cell count measurements after HIV viral load becomes undetectable and CD4+ T cell counts are >350/mm^3. We believe this limitation is unlikely to impact our results significantly, as once antiretroviral therapy (ART) is initiated, the CD4+ T cell count tends to remain stable or increase progressively, and even after virologic failure, CD4+ counts take months or years to drop to pre-ART levels.[25]

PLWH are historically more vulnerable to complications of common viral respiratory diseases such as influenza[26] but the interaction between HIV and SARS-CoV-2 is still unclear. Although some observational and epidemiological data suggest no greater risk of detrimental outcomes of COVID-19 among PLWH, especially among those with well-controlled HIV infection,[5,27] there are a few other studies that show higher mortality in PLWH compared to HIV-uninfected individuals.[28] Interestingly, studies from different epidemiological contexts support that race and schooling are associated with greater mortality among PLHIV with SARS-CoV-2 infection,[29] and social issues may overtake immune dysfunctions as determinants of COVID-19 outcomes in this population.

Our results showed that CoronaVac has robust immunogenicity in PLWH after a two-dose regimen, but antibody responses in this population are somewhat lower than in non-immunosuppressed individuals. Strategies should be developed to improve vaccine-induced immunogenicity in PLWH, especially in the subgroup with low CD4+ T cell counts. One possible approach is using a booster vaccine dose or even administering higher antigen titers per vaccine dose. Such strategies are already utilized among PLWH, *e.g.*, in Hepatitis B vaccination.[30]

Although this is the first controlled study analysing COVID-19 inactivated vaccine-induced immunogenicity among PLHIV, data on clinical efficacy and real-life effectiveness studies are still lacking for this population, with limited data so far from big vaccine developers. More than 38 million people are estimated to be living with HIV worldwide, with almost 1 million cases living in Brazil. With such an overlay of these two pandemics, it is essential to reinforce strategies to mitigate the damage caused by the SARS-CoV-2 pandemic in the already vulnerable HIV population.

Acknowledgements: We thank the study participants for their voluntary contribution. This study was sponsored by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (no. 2015/03756–4 to N.E.A., S.G.P., C.A.S.

and E.B.; no. 2017/14352-7 to T.P.) and from Conselho Nacional de Desenvolvimento Científico e Tecnológico (no. 305242/2019-9 to E.B.). Instituto Butantan supplied the study product and had no other role in the trial.

Author contributions:
EB, ACMR and EGK conceptualized the study. KYI, CMP, APPSA, EVA, MRS, PSSP, and ANL contributed with data collection and follow-up visits for PLWH. NEA, ACMR, SGP, EFNY, CGSS, TP, and CC contributed with data collection and follow-up visits for controls. VIAS performed statistical analysis. LCN, VIAS, EGK and EB wrote the manuscript. VIAS and LCN verified the underlying data. All author revised and approved the final version of the manuscript. All authors had full access to all the data in the study and accept responsibility to submit for publication.

Declaration of interests: EGK is the Principal Investigator for the CoronaVac phase 3 clinical trial at University of Sao Paulo. VIAS is the Principal Investigator for the Janssen COVID-19 vaccine phase 3 clinical trial at University of Sao Paulo.

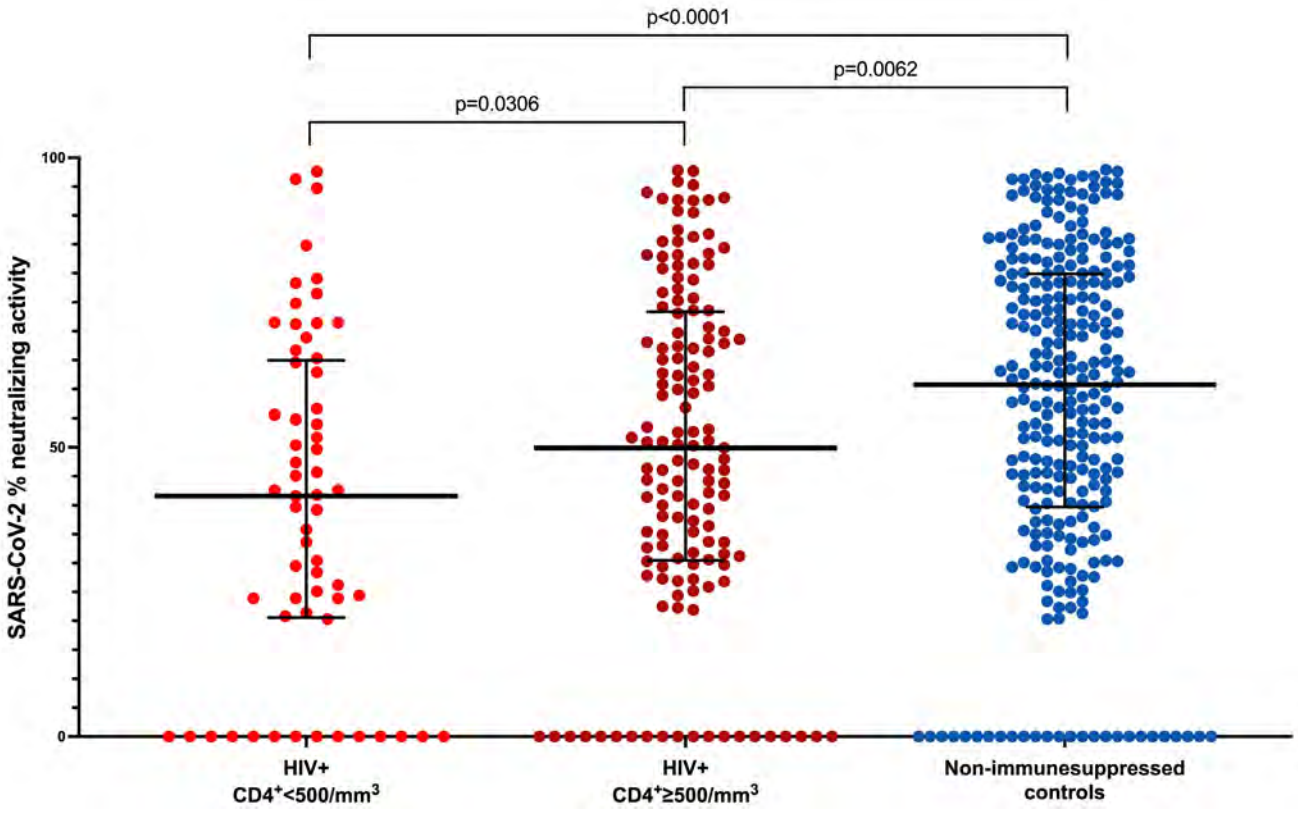
Data sharing statement: De-identified, individual participant data, a data dictionary defining each field in the dataset, study protocol and statistical analysis plan will be made available to others after the publication of this manuscript, following approval of a proposal. Proposals should be directed to esper.kallas@usp.br; to gain access, data requestors will need to sign a data access agreement.

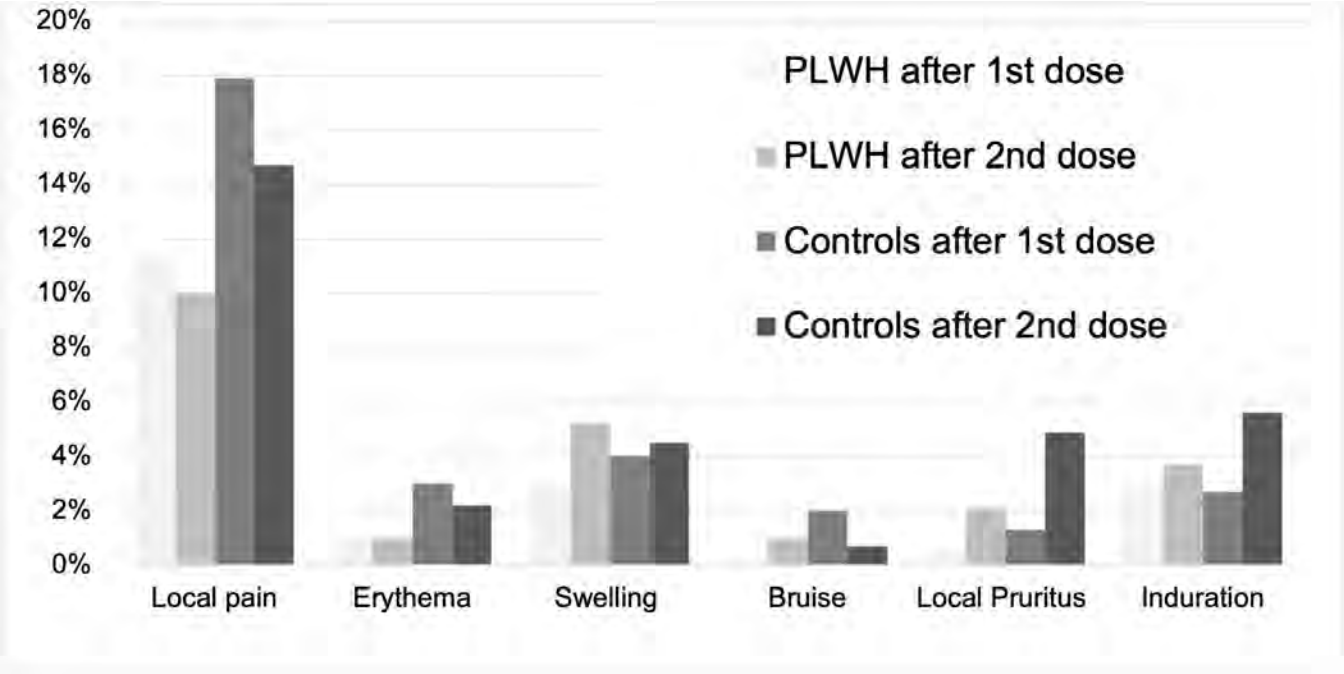
Uncategorized References

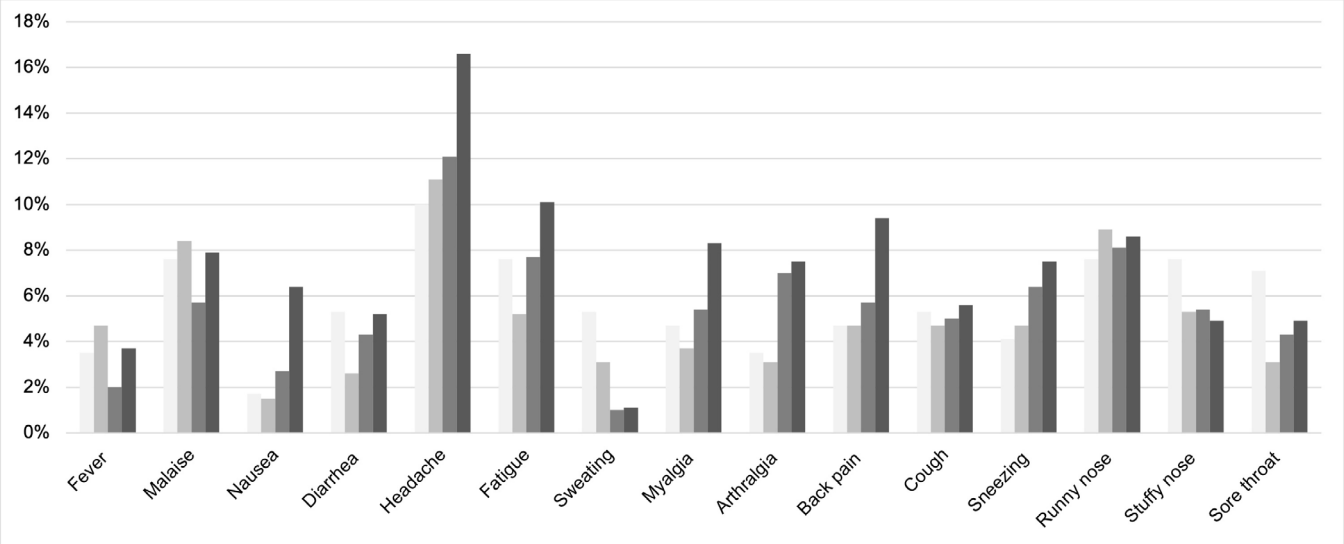
1. Castro MC, Gurzenda S, Turra CM, Kim S, Andrasfay T, Goldman N. Reduction in life expectancy in Brazil after COVID-19. *Nat Med* 2021; **27**(9): 1629-35.
2. Tanriover MD, Doganay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *Lancet* 2021; **398**(10296): 213-22.
3. Jara A, Undurraga EA, Gonzalez C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *N Engl J Med* 2021; **385**(10): 875-84.
4. England. PH. Guidance on shielding and protecting people who are clinically extremely vulnerable from COVID-19. *GovUk* 2020; **1**.
5. Ambrosioni J, Blanco JL, Reyes-Uruena JM, et al. Overview of SARS-CoV-2 infection in adults living with HIV. *Lancet HIV* 2021; **8**(5): e294-e305.
6. Geretti AM, Doyle T. Immunization for HIV-positive individuals. *Curr Opin Infect Dis* 2010; **23**(1): 32-8.

7. Avelino-Silva VI, Miyaji KT, Hunt PW, et al. CD4/CD8 Ratio and KT Ratio Predict Yellow Fever Vaccine Immunogenicity in HIV-Infected Patients. *PLoS Negl Trop Dis* 2016; **10**(12): e0005219.
8. Mahase E. Covid-19: Moderna applies for US and EU approval as vaccine trial reports 94.1% efficacy. *BMJ* 2020; **371**: m4709.
9. Ruddy JA, Boyarsky BJ, Werbel WA, et al. Safety and antibody response to the first dose of severe acute respiratory syndrome coronavirus 2 messenger RNA vaccine in persons with HIV. *AIDS* 2021; **35**(11): 1872-4.
10. Madhi SA, Koen AL, Izu A, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 in people living with and without HIV in South Africa: an interim analysis of a randomised, double-blind, placebo-controlled, phase 1B/2A trial. *Lancet HIV* 2021; **8**(9): e568-e80.
11. Butantan I. https://vacinacovid.butantan.gov.br/assets/arquivos/Bulas_Anvisa/.pdf. *Bula prossional da saúdepdf* 2021; **BLPFVCORFA_V08-21**.
12. Taylor SC, Hurst B, Charlton CL, et al. A New SARS-CoV-2 Dual-Purpose Serology Test: Highly Accurate Infection Tracing and Neutralizing Antibody Response Detection. *J Clin Microbiol* 2021; **59**(4).
13. Ghosn J, Taiwo B, Seedat S, Autran B, Katlama C. Hiv. *Lancet* 2018; **392**(10148): 685-97.
14. Grossman Z, Meier-Schellersheim M, Paul WE, Picker LJ. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. *Nat Med* 2006; **12**(3): 289-95.
15. Madhi SA, Maskew M, Koen A, et al. Trivalent inactivated influenza vaccine in African adults infected with human immunodeficient virus: double blind, randomized clinical trial of efficacy, immunogenicity, and safety. *Clin Infect Dis* 2011; **52**(1): 128-37.
16. Garrido HMG, Schnyder JL, Tanck MWT, et al. Immunogenicity of pneumococcal vaccination in HIV infected individuals: A systematic review and meta-analysis. *EClinicalMedicine* 2020; **29-30**: 100576.
17. Kerneis S, Launay O, Turbelin C, Batteux F, Hanslik T, Boelle PY. Long-term immune responses to vaccination in HIV-infected patients: a systematic review and meta-analysis. *Clin Infect Dis* 2014; **58**(8): 1130-9.
18. Medeiros-Ribeiro AC, Aikawa NE, Saad CGS, et al. Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial. *Nat Med* 2021.
19. Deepak P, Kim W, Paley MA, et al. Glucocorticoids and B Cell Depleting Agents Substantially Impair Immunogenicity of mRNA Vaccines to SARS-CoV-2. *medRxiv* 2021.
20. Haberman RH, Herati R, Simon D, et al. Methotrexate hampers immunogenicity to BNT162b2 mRNA COVID-19 vaccine in immune-mediated inflammatory disease. *Ann Rheum Dis* 2021; **80**(10): 1339-44.
21. Kamar N, Abravanel F, Marion O, Couat C, Izopet J, Del Bello A. Three Doses of an mRNA Covid-19 Vaccine in Solid-Organ Transplant Recipients. *N Engl J Med* 2021; **385**(7): 661-2.

22. Husain SA, Tsapepas D, Paget KF, et al. Postvaccine Anti-SARS-CoV-2 Spike Protein Antibody Development in Kidney Transplant Recipients. *Kidney Int Rep* 2021; **6**(6): 1699-700.
23. Miyaji KT, Avelino-Silva VI, Simoes M, et al. Prevalence and titers of yellow fever virus neutralizing antibodies in previously vaccinated adults. *Rev Inst Med Trop Sao Paulo* 2017; **59**: e2.
24. Fischinger S, Boudreau CM, Butler AL, Streeck H, Alter G. Sex differences in vaccine-induced humoral immunity. *Semin Immunopathol* 2019; **41**(2): 239-49.
25. Deeks SG, Barbour JD, Martin JN, Swanson MS, Grant RM. Sustained CD4+ T cell response after virologic failure of protease inhibitor-based regimens in patients with human immunodeficiency virus infection. *J Infect Dis* 2000; **181**(3): 946-53.
26. Sheth AN, Patel P, Peters PJ. Influenza and HIV: lessons from the 2009 H1N1 influenza pandemic. *Curr HIV/AIDS Rep* 2011; **8**(3): 181-91.
27. Cooper TJ, Woodward BL, Alom S, Harky A. Coronavirus disease 2019 (COVID-19) outcomes in HIV/AIDS patients: a systematic review. *HIV Med* 2020; **21**(9): 567-77.
28. Bhaskaran K, Rentsch CT, MacKenna B, et al. HIV infection and COVID-19 death: a population-based cohort analysis of UK primary care data and linked national death registrations within the OpenSAFELY platform. *Lancet HIV* 2021; **8**(1): e24-e32.
29. Rocha SQ, Avelino-Silva VI, Tancredi MV, et al. COVID-19 and HIV/AIDS in a cohort study in Sao Paulo, Brazil: outcomes and disparities by race and schooling. *AIDS Care* 2021: 1-7.
30. Launay O, van der Vliet D, Rosenberg AR, et al. Safety and immunogenicity of 4 intramuscular double doses and 4 intradermal low doses vs standard hepatitis B vaccine regimen in adults with HIV-1: a randomized controlled trial. *JAMA* 2011; **305**(14): 1432-40.


p<0.0001
p=0.0062
p=0.0306
SARS-CoV-2 % neutralizing activity
100
50
0
HIV+
CD4+<500/mm3
HIV+
CD4+≥500/mm3
Non-immunesuppressed
controls


20%
18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
PLWH after 1st dose
PLWH after 2nd dose
Controls after 1st dose
Controls after 2nd dose
Local pain
Erythema
Swelling
Bruise
Local Pruritus
Induration


18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
Fever
Malaise
Nausea
Diarrhea
Headache
Fatigue
Sweating
Myalgia
Arthralgia
Back pain
Cough
Sneezing
Runny nose
Stuffy nose
Sore throat

Comparing immune responses to inactivated vaccines against SARS-CoV-2 between people living with HIV and HIV-negative individuals: a cross-sectional study in China

Xiaojie Huang[1*], Ph.D.; Ying Yan[2*], Ph.D.; Bin Su[1*], Ph.D.; Dong Xiao[3*], Maohe Yu[4], B.A.; Xia Jin[5], M. S.; Junyi Duan[1], B.A.; Xiangjun Zhang[6], Ph.D.; Shimin Zheng[7], Ph.D.; Yuan Fang[8], Ph.D.; Weiming Tang[9#], Ph.D.; Lunan Wang[2#], Ph.D.; Tong Zhang[1#], Ph.D.; Zixin Wang[10#], Ph.D.; Junjie Xu[11#], Ph.D.

Affiliations:

[1] Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing, 100069, China.

[2] National Center for Clinical Laboratories, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing Hospital/National Center of Gerontology, 100044, China.

[3] Rainbow clinic of Beijing Jingcheng Skin Hospital, Beijing, 100101, China.

[4] Department of AIDS/STD Control and Prevention, Tianjin Centers for Disease Control and Prevention, Tianjin, 300011, China.

[5] AIDS Healthcare Foundation (AHF), Beijing, 100088, China.

[6] Department of Public Health, The University of Tennessee, Knoxville, Tennessee, 37996, USA.

[7] Department of Biostatistics and Epidemiology, East Tennessee State University, Johnson City, Tennessee, 70300, USA.

[8] Department of Early Childhood Education, The Education University of Hong Kong, Hong Kong, 200092, China.

[9] University of North Carolina Project-China, Guangzhou, Guangdong, 510095, China.

[10] JC School of Public Health and Primary Care, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, 666888, China.

[11] Clinical Research Academy, Peking University Shenzhen Hospital, Peking University, Shenzhen, 518036 China.

*, Co-first authors.

#, equal contribution as corresponding authors.

Corresponding author: Weiming Tang, Dermatology Hospital of Southern Medical University, Guangzhou, China; University of North Carolina Project-China, Guangzhou 510095, China. Email: Weiming_tang@med.unc.edu or;

Lunan Wang, National Center for Clinical Laboratories, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing Hospital/National Center of Gerontology, 100044, China. Email: lnwang@nccl.org.cn;

Tong Zhang, Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing, 100069, China. Email: zt_doc@ccmu.edu.cn;

Zixin Wang, JC School of Public Health and Primary Care Faculty of Medicine, The Chinese University of Hong Kong, Room 508, School of Public Health, Prince of Wales Hospital, Shatin, NT, Hong Kong, 666888, China. Email: wangzx@cuhk.edu.hk;

Junjie Xu, Clinical Research Academy, Peking University Shenzhen Hospital, Peking University, Shenzhen, 518036 China. Email:xjjbeijing@gmail.com;

Running head: Comparing immune responses to inactivated vaccines against SARS-CoV-2 between people living with HIV and HIV-negative individuals.

Conflict of Interest Disclosures: The authors have no conflicts of interest to disclose.

Funding/Support: This work was supported by the Beijing Excellent Talent Plan (2018000021223ZK04) , Beijing Talent Project in the New Millennium (2020A35), and the National Institute of Mental Health of the National Institutes of Health under Award (R34MH119963).

Summary

Background There are concerns about the efficacy and safety of SARS-Cov-2 vaccines among People living with HIV (PLWH). We compared immunogenicity and safety of the inactivated SARS-CoV-2 vaccines (Sinopharm and Sinovac CoronaVac) between PLWH and HIV-negative individuals.

Methods PLWH and HIV-negative individuals aged 18-59 years who had received at least one dose of inactivated SARS-CoV-2 vaccine were recruited in two Chinese cities between April and June 2021. Participants completed a self-administered questionnaire collecting adverse events and background charactersitics. Venous blood samples were collected and tested for neutralizing antibody responses against authentic SARS-CoV-2, the total antibody specific to SARS-CoV-2, SARS-CoV-2 IgG antibody against the receptor-binding domain of the spike protein (S-IgG), and antigen-specific T-cell immune response level.

Findings A total of 129 PLWH and 53 HIV-negative individuals completed this study. Prevalence (P=0·19) and severity (P=0·13-0·77) of adverse events were similar among PLWH and HIV-negative individuals. The prevalence of seropositivity of neutralizing antibody, total antibody and S-IgG was 71·3%, 81·9% and 92·5% among fully vaccinated PLWH, which is similar to fully vaccinated HIV-negative individuals (P=0·07-0·48). Among all participants, PLWH had significantly lower neutralizing antibody, total antibody, S-IgG, and T-cell specific immune response levels compared to HIV-negative individuals, after controlling for types of vaccine, time interval between prime and second dose, time after receiving the second dose, and sociodemographics. PLWH who had a longer time since HIV diagnosis, completed the second dose for 15-28 days, and an interval between prime and second dose of ≥21 days had higher neutralizing antibody levels.

Intrepretation Inactivated SARS-CoV-2 vaccines are safe for PLWH. Fully vaccinated PLWH could achieve similarly high protection as HIV-negative individuals. Vaccination guidelines for PLWH should be developed.

Funding Beijing Excellent Talent Plan, Beijing Talent Project in the New Millennium, the National Institute of Mental Health of the National Institutes of Health under Award.

Keywords: People living with HIV; Inactivated SARS-CoV-2 vaccines; self-reported adverse events; neutralizing antibody responses against authentic SARS-CoV-2; total antibody specific to SARS-CoV-2; SARS-CoV-2 IgG antibody; antigen-specific T-cell immune response.

Introduction

Globally, about 38 million people are living with HIV [1]. Antiretroviral therapy (ART) could suppress viral replication, restore $CD4^+$ T-cell counts, rebuild immune function, and decrease morbidity and mortality among people living with HIV (PLWH) [2,3]. However, $CD4^+$ T-cell recovery is incomplete despite viral suppression in some PLWH [4]. The World Health Organization (WHO) confirmed that HIV infection is a significant independent risk factor for both severe SARS-CoV-2 cases at hospital admission and in-hospital mortality [5]. Both international health authorities and Chinese national guidelines recommend SARS-CoV-2 vaccination to PLWH regardless of their immune status [6-8].

PLWH is considered a priority group for vaccination in many countries [8]. However, there are concerns that PLWH might have a suboptimal response to SARS-CoV-2 vaccination. More imporntantly, less than 3% of the participants in the reported SARS-CoV-2 vaccine efficacy trials are PLWH, and the data for vaccine safety and immune response is insufficient [9-13]. The Novarax study showed the overall vaccine efficacy was higher when excluding PLWH from the analysis (increased from 49·4% to 60%) [13]. Most studies did not report vaccine efficacy specific for PLWH. Some studies have compared the safety and immunogenicity of mRNA (Pfizer BNT162b2 and Moderna mRNA-1273) or adenovirus vector (Oxford/AstraZeneca AZD1222) SARS-CoV-2 vaccines between HIV-negative individuals and PLWH with viral suppression and high $CD4^+$ T-cell levels (median around 700) [14-18]. These studies showed that SARS-CoV-2 vaccines were safe for PLWH, and there was no between-group difference in adverse events [14-18].

There are two inactivated SARS-CoV-2 vaccines manufactured by Chinese companies are approved for emergency use by the WHO (Sinopharm and Sinovac CoronaVac) [19,20]. More than three billion doses of these vaccines has been supplied to more than 40 countries [21]. No study compared PLWH and HIV-negative individuals regarding immunogenicity and safety of the inactivated SARS-CoV-2 vaccines. Such evidence is important to address COVID-19 vaccine hesitancy among PLWH or to implement boost dose for this group [22]. Previous findings on mRNA/adenovirus vector vaccines might not be applicable to PLWH receiving inactivated SARS-CoV-2 vaccines [14-18]. Moreover, it is unclear whether PLWH with lower $CD4^+$ T cell counts and detectable HIV viral load would have similar immunogenicity as HIV-negative individuals, as these PLWH were excluded by the aforementioned studies [14-18]. Furthermore, given the relatively short follow-up period in previous studies, there is no

consensus about the long-term immunogenicity to SARS-CoV-2 vaccines among PLWH [14-18].

This study aims to address these knowledge gaps by comparing the immunogenicity and adverse events between PLWH and HIV-negative individuals after vaccination. This study also investigated factors correlated with levels of neutralizing antibody responses against authentic SARS-CoV-2, the total antibody specific to SARS-CoV-2, SARS-CoV-2 IgG antibody against the receptor-binding domain (RBD) of the spike protein (S-IgG), and antigen-specific T-cell immune response among PLWH.

Methods

Study design

This cross-sectional study was conducted in two Chinese metropolitan cities (Beijing and Tianjin) conducted between April and June 2021. Participants included PLWH and HIV-negative individuals who have received at least one dose of inactivated SARS-Cov-2 vaccine.

Participants

The inclusion criteria for PLWH included: 1) aged 18-59 years, 2) willing to participate in the study activities, including survey and blood sample collection, and relevant laboratory testing, 3) having received at least one dose of inactivated SARS-CoV-2 vaccine (Sinovac CoronaVac or Sinopharm), and 4) having received HIV diagnosis confirmed by HIV-1/2 western blot assay. Exclusion criteria included: 1) presence of severe hearing loss, impaired vision, or intellectual disability observed by the interviewers, and 2) history of SARS-CoV-2 infection, major psychiatric illness (schizophrenia and bipolar disorder) or neurocognitive impairment based on clinician's assessment of their medical records. HIV-negative individuals shared the first three inclusion criteria and both exclusion criteria with PLWH. HIV serostatus was confirmed by Abbott ARCHITECT HIV Ag/Ab Combo assay.

Recruitment and data collection

Recruitment for PLWH was facilitated by two community-based organizations (CBOs), one in each city. These two CBOs have provided services to PLWH and HIV high-risk populations and worked closely with HIV clinical service providers. WeChat is the most commonly used social media application for the CBOs to communicate with PLWH clients. CBO staff posted the study recruitment information in the WeChat public accounts of their organizations. Interested PLWH contacted CBO staff through private WeChat messages, phone calls, and messages via other instant messaging applications. CBO staff screened

participants' eligibility, briefed them about the study purpose and procedures, assured them that identifiable information would be kept confidential, and refusal to participate would have no consequences. The recruitment of HIV-negative individuals was conducted in community hospitals. The hospital staff approached vaccinated individuals in their service records by telephone and invited them to participate.

PLWH and HIV-negative individuals interested in joining the study were invited to visit one of two clinics, one in each city. On-site, project staff obtained their written informed consent. All participants completed a 10-minute self-administered questionnaire on site. The STROBE checklist was adhered (see Appendix).

Blood sample collection and laboratory procedures

After completion of the survey, trained nurses collected two lithium heparin anticoagulated vacuum blood collection tubes (BD) of whole blood (10 ml), two EDTA anticoagulated vacuum blood collection tubes (BD) of whole blood (10 ml), and one SST blood collection tube of whole blood (5ml). One tube of lithium heparin salt anticoagulated whole blood and one tube of EDTA anticoagulated whole blood were placed at room temperature. They were assayed for T cell-specific immune response within 8 hours and $CD4^+$ T-cell count within 48 hours, respectively. The other three tubes of whole blood were centrifuged at 1300 relative centrifugal force (RCF) for 10 minutes, and the upper plasma/serum layers were transferred into lyophilized tubes of no less than 1·2 ml each, and were stored at -20°C for the detection of SARS-Cov-2 combined antibody and neutralizing antibody, as well as HIV viral load.

SARS-CoV-2 neutralizing antibody measurement. The neutralizing antibodies to authentic SARS-CoV-2 (virus strain SARS-CoV-2/human/CHN/CN1/2020, GenBank number MT407649.1) were quantified using a micro cytopathogenic effect (CPE) inhibition assay with a minimum four-fold dilution as reported before [23]. The positive geometric mean titer (GMT) of the neutralizing antibodies to authentic SARS-CoV-2 was 8.

SARS-CoV-2 antigen/antibody combined testing. All samples were tested for total antibody and SARS-CoV-2 specific S-IgG antibodies using Chemiluminescence assay (CLIA) kits (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.). The positive cut-off for the abovementioned tests was 1·0.

T-cell specific immune response. The T cell specific immune response was tested using the IFN-γ release assay (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.). Briefly, 1·5 ml of heparin blood was distributed into test tube containing specific SARS-CoV-2 S antigen (T tube), negative control tube (N tube), and positive control tube (P tube) within 8 hours.

The tubes were inverted and mixed 5 times, incubated in 37℃ for 20-24 hours. Then the plasma was collected after centrifuging at 3000 RCF for 10 minutes and detected for IFN-γ level. Level of T tube minus N tube, a value greater than 30 pg/ml was considered positive.

HIV viral load detection Viral load of PLWH was tested using HIV quantitative assay (Zhuhai Livzon Diagnostics Inc.). The limit of quantitation (LOQ) of this assay was 60 copies/ml.

$CD4^+$ cell count measurement. The assay was performed using flow cytometry testing methods (BD Biosciences, San Jose, CA, USA) in accordance with the China National Guideline for Detection of HIV/AIDS (version 2020) [24].

Background characteristics of the participants. All participants reported age, gender, and presence of chronic conditions. Characteristics related to HIV infection and SARS-CoV-2 vaccination were extracted from medical records.

Adverse events related to SARS-CoV-2 vaccination. A checklist was used to assess local adverse events (pain, redness, itch, swelling, induration, and skin rash in the arm where the shot was given) and systematic adverse events (fatigue, malaise, headache, dizziness, lethargy, joint pain or muscle ache, feverish, nausea, vomit, diarrhea, and others) within one month after receiving SARS-CoV-2 vaccines. Participants rated the severity the aforementioned adverse events (1=very mild, 2=mild, 3=moderate, 4=severe, and 5=very severe).

Sample size planning

Previous studies showed that the positive rate for SARS-CoV-2 neutralizing antibody was about 90% among HIV-negative individuals who received inactivated SARS-CoV-2 vaccines [23]. There was no data on seropositivity for SARS-CoV-2 neutralizing antibody among PLWH who received inactivated vaccines. Previous studies showed that the seroconversion rate of PLWH after inoculation of the hepatitis B vaccine ranged from 34% to 88% [25]. Therefore, we assumed 70% of vaccinated PLWH would be positive for SARS-CoV-2 neutralizing antibody. Using an allocation ratio of 2:1, a total of 102 PLWH and 51 HIV-negative individuals was required to detect a minimum between-group difference of 20% (90% versus 70%) in SARS-CoV-2 neutralizing antibody positive rate (α= 0·05, β= 0·10).

Statistical analysis

Chi-square tests were used to inspect the difference in background characteristics and adverse events related to SARS-CoV-2 vaccination between PLWH and HIV-negative individuals. Between-group differences in immunogenicity indicator levels (total antibody, neutralizing

antibody, S-IgG, and T-cell specific immune response) were tested using Mann-Whitney tests. We log transformed the immunogenicity indicator levels using the base of 10 to normalize the data. Multivariable linear regression models were performed to test the between-group difference in these indicators, after controlling for all background characteristics with $P<0{\cdot}05$ in between-group comparisons. Adjusted coefficients (B) were obtained. Moreover, same comparisons were performed between different subgroups of PLWH and HIV-negative individuals. Similar analyses on sero-positivity for these immunogenicity indicators was also performed. Among PLWH, linear regression models were used to inspect factors that were correlated with immunogenicity indicator levels. SPSS version 26.0 was used in all analyses, with two-tailed $P<0{\cdot}05$ was considered statistically significant.

Ethics approval

Written informed consent was obtained from all participants before their study participation in accordance with the Declaration of Helsinki. The Institutional Review Boards of Changzhi Medical College (RT2021002) and Beijing Youan Hospital Research Ethics Committee (No. 2021-031) approved this study.

Role of funding sources

This study was supported by the Beijing Excellent Talent Plan (2018000021223ZK04), the Beijing Talent Project in the New Millennium (2020A35), the National Natural Science Foundation of China (81772165 and 81974303 to B.S.), the National 13th Five-Year Grand Program on Key Infectious Disease Control (2017ZX10202102-005-003 to B.S.), and the China Primary Health Care Foundation-Youan Medical Development Fund (BJYAYY-2020PY-01 to B.S.), and the Beijing Key Laboratory for HIV/AIDS Research (BZ0089). Funders had no role in the design, data collection, analysis, interpretation of the study, or the preparation of the manuscript.

Results

Profiles of the participants

A total of 519 and 316 PLWH in Beijing and Tianjin were approached, 130 and 24 were screened to be eligible, and 110 (84·6%) and 19 (79%) completed the study. At the same period, 61 vaccinated HIV-negative individuals were approached, 8 (13·1%) refused to participate mainly due to logistic reasons, and 53 (86·9%) completed the study procedures.

Most PLWH received HIV diagnoses for more than one year (86%), and were on ART (97·7%). Over half of them had an undetectable viral load (58·1%), and the median $CD4^+$ T-cell count was 630·5 (IQR: 499·5, 848·8) (Table 1).

As compared to HIV-negative individuals, fewer PLWH were 50-59 years old (3·9% versus 17·0%, *P*=0·01) and female (0·8% versus 24·5%, *P*<0·001). More PLWH had chronic conditions (20·9% versus 0%, *P*<0·001), received Sinovac-CoronaVac (55·0% versus 30·2%, *P*<0·001) and only completed the prime dose (27·1% versus 3·8%, *P*<0·001). Receiving more than one type of vaccine was not observed. Among those who completed both doses, the time interval between the prime and second dose was shorter among PLWH than HIV-negative individuals were (median: 21 versus 27 days, *P*<0·001) (Table 1). These background characteristics were controlled when comparing immunogenicity indicators levels between PLWH and HIV-negative individuals.

SARS-CoV-2 vaccination adverse events

Among the participants, 45·0% of PLWH and 54·7% of HIV-negative individuals reported presence of any specific local and systematic adverse events. After controlling for significant background characteristics (i.e., age group, gender, presence of chronic conditions other than HIV, types of vaccine, time interval between prime and second dose, and time after receiving the second dose), there is no between-group difference in prevalence of any adverse events (AOR: 0·77, 95%CI: 0·31, 1·95, *P*=0·19). Most of the reported adverse events were very mild/mild (41-100% among PLWH and 62·2-100% among HIV-negative individuals). There was no between-group difference in the severity of these adverse events (*P*=0·13-0·77). (Table 2)

Subgroup analysis showed that PLWH did not have a higher prevalence of any adverse events when comparing with HIV-negative individuals, regardless of $CD4^+$ T-cell counts or HIV viral suppression status (Appendix 1).

Immunogenicity indicators level

The prevalence of seropositivity of neutralizing antibody, total antibody and S-IgG was 71·3%, 81·9% and 92·5% among fully vaccinated PLWH. Such prevalence is similar to that observed among fully vaccinated HIV-negative individuals (*P*=0·07-0·48). (Appendix 2). When comparing to HIV-negative individuals, PLWH had significantly lower levels of neutralizing antibody (adjusted B: -0·18, *P*=0·049), total antibody (adjusted B: -0·80, *P*<0·001), S-IgG (adjusted B: -0·31, *P*=0·002), and T-cell specific immune response (adjusted B: -0·64, *P*=0·002). Subgroup analyses showed that PLWH with detectable viral load (adjusted B: -0·29, *P*=0·047) or $CD4^+$ T cell counts <500 (adjusted B: -0·29, *P*=0·02)

had significantly lower neutralizing antibody levels. Such difference in neutralizing antibody level was not observed when comparing HIV-negative individuals with PLWH with undetectable viral load or $CD4^+$ T cell counts ≥500. In addition, PLWH had significantly lower levels of total antibody, S-IgG, and T-cell specific immune response regardless of $CD4^+$ T cell counts or HIV viral suppression. Neutralizing antibody levels among fully vaccinated PLWH did not lower than fully vaccinated HIV-negative individuals (adjusted B: -0·15, *P*=0·13) (Table 3 & 4).

Factors associated with immunogenicity indicator levels among PLWH

A longer time since HIV diagnosis was associated with higher neutralizing antibody and total antibody levels (2-5 years: adjusted B: 0·71 & 0·27; reference: ≤1 year). As compared to partially vaccinated participants, PLWH who completed the second dose for 15-28 days had higher neutralizing antibody levels (adjusted B: 0·30), while those who completed it for 15-56 days had higher total antibody (adjusted B: 1·00), S-IgG (adjusted B: 0·53), and T-cell specific immune response levels (adjusted B: 0·89-0·99). Compared to PLWH with a time interval of <21 days between the prime and second dose, those with an interval of 21-28 days and >28 days had higher neutralizing antibody (adjusted B: 0·37 & 0·36), total antibody (adjusted B: 1·22 & 1·28), and S-IgG levels (adjusted B: 0·43 & 0·53) (Table 5).

Discussion

Understanding the differences of immunoresponse between HIV negative and positive individuals is essential in planning the SARS-CoV-2 vaccination for PLWH. We found the levels of adverse events are comparable between PLWH and HIV-negative individuals. The prevalence of seropositivity of neutralizing antibody, the total antibody, and S-IgG were similarly high among fully vaccinated PLWH and HIV-negative individuals. However, PLWH had lower immunogenicity indicator levels than HIV-negative individuals after controlling for types of vaccine, time since receiving the prime dose, time interval between prime and second dose, and socio-demographics. Our findings filled the knowledge gap on the immune responses to SARS-CoV-2 vaccines among PLWH. It contributed critical evidence to policymaking and vaccination program planning for countries that mainly using inactivated SARS-CoV-2 vaccines.

Similar to studies on mRNA/adenovirus vector SARS-CoV-2 vaccines [14-18], there was no between-group difference in prevalence (*P*=0·19) or severity (*P*=0·13-0·77) of self-reported

adverse events. Most of the reported adverse events were very mild/mild among PLWH (41-100%). Therefore, inactivated SARS-CoV-2 vaccines are safe for PLWH.

Four immunogenicity indicator levels were significantly lower among PLWH at 0-14 days after receiving the second dose. PLWH might take longer to develop humoral and cellular immune responses to inactivated SARS-CoV-2 vaccines. Previous case reports observed a prolonged course of antibody development among PLWH infected with SARS-CoV-2 [26]. Similar to HIV-negative individuals and PLWH who received other SARS-CoV-2 vaccines, the studied immunogenicity indicators peaked at 15-56 days after the second dose among PLWH [14-18]. However, the peak levels of these indicators were lower among PLWH, especially for total antibody and S-IgG. A faster decline in immune responses were also observed among PLWH. All four immunogenicity indicators levels declined >56 days after receiving the second dose among PLWH, while these indicators remained stable among HIV-negative individuals even 84 days after the second dose. This study observed significantly lower total antibody and S-IgG levels among PLWH >56 days after the second dose. B-cell dysfunction caused by HIV gp120 binds directly to primary B-cell, and impaired cellular immunity caused by $CD4^+$ T cell depletion among PLWH might explain slower development, lower peak levels, and faster decline of both humoral and cellular immune responses to SARS-CoV-2 vaccines [27,28]. Such findings indicated that PLWH might need a boost dose after the initial doses, and might need it earlier than HIV-negative individuals do. Future studies with large sample size are needed to investigate long-term changes in these immunogenicity indicators among PLWH.

Neutralizing antibody plays an important role in SARS-CoV-2 clearance and is a key indicator for protection after vaccination [29]. We found that the seropositivity and levels of neutralizing antibody was similarly high among fully vaccinated PLWH and HIV-negative individuals. It implied that both groups obtained good protection against SARS-Cov-2 after the vaccination and PLWH should complete both doses of vaccination as required. Subgroup analysis showed that in line with studies using mRNA and/or adenovirus vector SARS-CoV-2 vaccines, PLWH with higher $CD4^+$ T-cell counts or undetectable viral load did not had significantly lower neutralizing antibody level than HIV-negative individuals [14-18]. However, PLWH with lower $CD4^+$ T-cell counts (<500) or detectable viral load had lower neutralizing antibody level. Such findings added knowledge to immune responses to SARS-CoV-2 vaccines among PLWH with severer immunodeficiency. PLWH with severer immunodeficiency should be encouraged to receive SARS-CoV-2 vaccines. In contrast to findings on other types of vaccines, our study observed significant lower total antibody, S-

IgG, and T-cell specific immune responses levels among PLWH compared to HIV-negative individuals. The difference could not be fully explained by the larger proportion of PLWH with low CD4+ T-cell counts or detectable HIV viral load in this study. These indicators were lower among PLWH regardless of their $CD4^+$ T-cell counts or HIV viral load. Future studies should compare PLWH's immunogenicity to different types of SARS-CoV-2 vaccines in order to determine the optimal choice for PLWH.

Compared to newly diagnosed PLWH, those who had been diagnosed for 2-5 years had higher neutralizing antibody and total antibody levels. It is possible that these PLWH had better functioning immune system after years of ART. It also highlighted the needs to further increase HIV testing coverage among key population to early identify HIV infection and link them to treatment and care. It will hence improve the effectiveness of SARS-CoV-2 vaccination for PLWH. Moreover, our results also suggested that, PLWH had a longer interval between the prime and second dose (21-28 days or >28 days) had significantly higher neutralizing antibody, total antibody and S-IgG levels compared to those with a shorter interval. Existing guidelines of SARS-CoV-2 vaccination for PLWH did not mention the optimal vaccination interval. Our findings suggested that future SARS-CoV-2 vaccination program for PLWH should consider a longer interval between doses. More research is needed to determine an optimal interval between doses for PLWH.

The study has several strengths. First, all participants underwent humoral and cellular immune responses analysis in this study. Second, this study included a diverse sample of PLWH with different $CD4^+$ T cell level and HIV viral load. It filled the knowledge gaps about immunogenicity to SARS-CoV-2 vaccines among PLWH with impaired functional immune system and poorer control of HIV. Third, impact of between-group difference in background characteristics on immunogenicity might be limited in this study, as background characteristics were controlled during the comparison. Furthermore, this is also one the first studies that assessed relationships between characteristics of PLWH and immunogenicity to SARS-CoV-2 vaccines.

This study also has some limitations. First, this was a cross-sectional study. Possible changes in immunogenicity indicator levels over time were unclear. Such study design cannot establish causal relationship as well. Second, we did not use matching to sample HIV-negative individuals according to PLWH's characteristics. There are significant between-group differences in socio-demographics, presence of other chronic conditions, and vaccination characteristics. We controlled these characteristics when comparing the between-group difference in immunogenicity. Third, PLWH was over-represented by male. However,

the impact of gender difference on immunogenicity might be limited, as previous studies did not show difference in immunogenicity between male and female [23]. Moreover, the presence and severity of adverse events were self-reported by participants and might be subject to recall bias. We were not able to compare the safety data with other studies that used clinician assessments.

Inactivated SARS-CoV-2 vaccines are safe for PLWH. Fully vaccinated PLWH could achieve similarly high protection as HIV-negative individuals. PLWH had significantly lower neutralizing antibody, total antibody, S-IgG, and T-cell specific immune response levels than HIV-negative individuals did. The immunogenicity indicator levels peaked 15-56 days after PLWH receiving the second dose. A longer time since diagnosis and a longer interval between the prime and second dose were correlated with better immune responses among PLWH. Future studies should compare PLWH's immunogenicity to different types of vaccines, assess immune responses in a longer term, and investigate the optimal interval between doses.

Contributors

All authors contributed to the conception of this study. XJH, WMT, and JJX developed the methodology. DX, YY, XJ, JYD, MHY, LNW, and JJX were responsible for site survey and coordination. XJH, BS, TZ, YY, LNW, and JJX were responsible for the laboratory testing and test result interpletation. WMT, ZXW, XJZ, SMZ, YF, and JJX wrote the original draft. All authors contributing to the reviewing and editing process. All authors agreed to submit the manuscript for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The individual participant data used in this analysis are available upon request. Requests should ne directed to the corresponding author, and need to sign a data access and confidentiality agreement.

Acknowledgments

The authors gratefully acknowledge the participation from HIV-negative individuals and PLWH who contribute to this research in the mainland of China. We are also grateful to local communities for the onsite recruitment and coordination.

414 **Table 1 Background characteristics of HIV-negative individuals and People living with HIV (PLWH) who had received at least one**
415 **dose of SARS-CoV-2 vaccine**

	People living with HIV (n=129)	HIV-negative individuals (n=53)	*P* values
Socio-demographics			
Age (years), n (%)			
18-29	39 (30·2)	14 (26·4)	
30-39	65 (50·4)	19 (35·8)	
40-49	20 (15·5)	11 (20·8)	
50-59	5 (3·9)	9 (17·0)	0·01
Median (IQR), range	34 (28, 38) (20-58)	34 (29, 47) (22-56)	0·15
Gender, n (%)			
Male	128 (99·2)	40 (75·5)	
Female	1 (0·8)	13 (24·5)	<0·001
Presence of chronic conditions other than HIV/AIDS			
No	102 (79·1)	53 (100·0)	
Yes	27 (20·9)	0 (0·0)	<0·001
Characteristics related to HIV infection			
Time since HIV diagnosis (years)			
≤1	18 (14·0)	N.A	N.A.
2-5	55 (42·6)	N.A	N.A.
6-10	35 (27·1)	N.A	N.A.
>10	21 (16·3)	N.A	N.A.
Viral load (cp/ml), n (%)			
Undetectable (≤60)	75 (58·1)	N.A.	N.A.
61-200	33 (25·6)	N.A.	N.A.
>200	21 (16·3)	N.A.	N.A.
CD4+ T cell count (cells/μL)			
<500	32 (24·8)	N.A.	N.A.
500-1,000	81 (62·8)	N.A.	N.A.
>1,000	16 (12·4)	N.A.	N.A.
Median (IQR), range	630·5 (499·5, 848·8) (78, 2650·35)	N.A.	N.A.
ART regimens			
TDF+3TC+EFV	60 (52·7)	N.A.	N.A.
TDF+3TC+LPV/r	5 (3·9)	N.A.	N.A.
AZT+3TC+LPV/r	3 (2·3)	N.A.	N.A.
AZT+3TC+NVP	2 (1·6)	N.A.	N.A.
AZT+3TC+EFV	8 (6·2)	N.A.	N.A.
Others	40 (31·0)	N.A.	N.A.
Not on ART	3 (2·3)	N.A.	N.A.
Information related to SARS-CoV-2 vaccination			

SARS-CoV-2 vaccination status			
Partially vaccinated	35 (27·1)	2 (3·8)	
0-14 days after fully vaccinated	15 (11·6)	8 (15·1)	
15-28 days after fully vaccinated	38 (29·5)	13 (25·5)	
29-56 days after fully vaccinated	26 (20·2)	21 (39·6)	
57-84 days after fully vaccinated	12 (9·3)	3 (5·7)	
>84 days after fully vaccinated	3 (2·3)	8 (15·1)	<0·001
Type of SARS-CoV-2 vaccine			
Sinopharm	58 (45·0)	37 (69·8)	
Sinovac-CoronaVac	71 (55·0)	16 (30·2)	<0·001
Time interval between the prime (1st) and second dose (among those who were fully vaccinated)	n=94	n=51	
<21 days	20 (21·3)	3 (5·7)	
21-28 days	58 (61·7)	40 (75·5)	
>28 days	16 (17·0)	10 (18·9)	0·043
Median (IQR), range	21 (21, 27) (14-59)	27 (21, 28) (14-83)	0·002

N.A.: not applicable.

418 **Table 2 Comparing self-reported local and systematic adverse events related to SARS-CoV-2 vaccination among People living with**
419 **HIV (PLWH) and HIV-negative individuals**

	People living with HIV (n=129)	HIV-negative individuals (n=53)	*P* values
	n (%)	n (%)	
Local adverse events			
Pain			
None	87 (67·4)	31 (58·5)	
Very mild	15 (11·6)	4 (7·5)	
Mild	16 (12·4)	11 (20·8)	
Moderate	11 (8·5)	7 (13·2)	
Severe	0 (0·0)	0 (0·0)	0·30
Any of above	42 (32·6)	22 (41·5)	0·25
Redness, itch, swelling, induration and/or skin rash			
None	124 (96·1)	50 (94·3)	
Very mild	0 (0·0)	1 (1·9)	
Mild	2 (1·6)	2 (3·8)	
Moderate	3 (2·3)	0 (0·0)	
Severe	0 (0·0)	0 (0·0)	0·21
Any of above	5 (3·9)	3 (5·7)	0·59
Systematic adverse events			
Fatigue, malaise, headache, dizziness, and/or lethargy			
None	107 (82·9)	43 (81·1)	
Very mild	5 (3·9)	3 (5·7)	
Mild	11 (8·5)	4 (7·5)	
Moderate	5 (3·9)	2 (3·8)	
Severe	1 (0·8)	1 (1·9)	0·94
Any of above	22 (17·1)	10 (18·9)	0·77
Joint pain and/or muscle ache			
None	119 (92·2)	45 (84·9)	
Very mild	4 (3·1)	1 (1·9)	
Mild	3 (2·3)	4 (7·5)	
Moderate	3 (2·3)	3 (5·7)	
Severe	0 (0·0)	0 (0·0)	0·23
Any of above	10 (7·8)	8 (15·1)	0·13
Fever			
None	122 (94·6)	52 (98·1)	
Very mild	2 (1·6)	0 (0·0)	
Mild	4 (3·1)	1 (1·9)	
Moderate	1 (0·8)	0 (0·0)	
Severe	0 (0·0)	0 (0·0)	0·69
Any of above	7 (5·4)	1 (1·9)	0·27
Nausea, vomit, and/or diarrhea			

None	129 (100·0)	52 (98·1)	
Very mild	0 (0·0)	0 (0·0)	
Mild	0 (0·0)	1 (1·9)	
Moderate	0 (0·0)	0 (0·0)	
Severe	0 (0·0)	0 (0·0)	0·12
Any of above	0 (0·0)	1 (1·9)	0·29
Other systematic side-effects			
None	127 (98·4)	53 (100·0)	
Very mild	2 (1·6)	0 (0·0)	
Mild	0 (0·0)	0 (0·0)	
Moderate	0 (0·0)	0 (0·0)	
Severe	0 (0·0)	0 (0·0)	0·36
Any of above	2 (1·6)	0 (0·0)	0·50
Any local and/or systematic adverse events	58 (45·0)	29 (54·7)	0·23

420

Table 3 Levels of SARS-CoV-2 neutralizing antibody, total antibody, S-IgG, and T cell specific immune response among HIV-negative individuals and people living with HIV (PLWH) who had received at least one dose of SARS-CoV-2 vaccine

	Neutralizing antibody			Total antibody			S-IgG			T cell specific immune response		
	PLWH	HIV-negative	*P*	PLWH	HIV-negative	*P*	PLWH	HIV-negative	*P*	PLWH	HIV-negative	*P*
	GMT (95%CI)	GMT (95%CI)		Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
Partially vaccinated	4·6 (4·0, 9·8)	5·6 (N.A.)	0·43	0·2 (0·02, 1·1)	2·1 (N.A.)	0·20	0·6 (0·3, 1·5)	3·99 (N.A.)	0·03	6·4 (0·2, 26·7)	36·2 (N.A.)	0·16
0-14 days after fully vaccinated	8·5 (4·0, 64·6)	31·6 (4·0, 257·0)	0·03	0·8 (0·03, 16·8)	104·8 (7·4, 279·5)	0·01	3·1 (1·1, 16·2)	11·9 (5·1, 55·5)	0·04	5·3 (0·1, 88·8)	413·6 (91·8, 575·5)	0·001
15-28 days after fully vaccinated	24·0 (4·0, 380·2)	23·4 (4·0, 64·0)	0·97	28·9 (7·4, 83·2)	40·3 (28·5, 71·6)	0·24	9·0 (4·6, 16·0)	13·9 (10·1, 32·0)	0·13	56·08 (19·6, 118·7)	91·54 (31·1, 227·4)	0·29
29-56 days after fully vaccinated	14·1 (4·0, 64·6)	20·9 (4·0, 190·5)	0·24	11·8 (5·7, 27·3)	42·7 (8·4, 74·9)	0·04	7·2 (4·5, 12·2)	9·6 (7·2, 21·9)	0·03	37·2 (6·4, 121·1)	63·6 (35·4, 182·1)	0·13
57-84 days after fully vaccinated	11·0 (4·0, 95·5)	26·3 (12·0, 64·0)	0·18	6·2 (0·5, 11·7)	33·4 (N.A.)	0·04	3·4 (1·4, 5·7)	10·5 (N.A.)	0·03	3·6 (0·1, 17·1)	205·5 (N.A.)	0·08
>84 days after fully vaccinated	6·3 (4·0, 8·0)	11·1 (4·0, 48·0)	0·50	3·0 (1·3, N.A.)	9·3 (4·0, 62·8)	0·15	3·8 (1·2, N.A.)	4·3 (2·9, 5·4)	0·31	18·3 (0·8, N.A.)	35·6 (13·5, 56·2)	0·41
Among all participants	11·0 (4·0, 95·5)	20·0 (4·0, 190·5)	0·001	5·6 (0·4, 25·2)	32·6 (8·4, 72·3)	<0·001	4·3 (1·2, 10·0)	9·6 (5·4, 18·9)	<0·001	18·7 (2·4, 77·9)	63·6 (36·0, 226·4)	<0·001
Among participants who were fully vaccinated	15·1 (4·0, 128·8)	20·9 (4·0, 190·5)	0·09	10·3 (2·3, 38·8)	33·4 (10·1, 73·0)	<0·001	6·8 (3·3, 12·1)	10·1 (6·5, 19·4)	0·007	30·6 (5·2, 103·2)	68·4 (36·1, 227·4)	0·001

P values were obtained by using Mann-Whitney tests.
N.A.: not applicable.

Table 4 Comparing immunogenicity indicator levels between different subgroups of people living with HIV (PLWH) and HIV-negative individuals

	Neutralizing antibody		Total antibody		S IgG		T cell specific immune response	
	Adjusted B (95%CI)	**P values**	**Adjusted B (95%CI)**	**P values**	**Adjusted B (95%CI)**	**P values**	**Adjusted B (95%CI)**	**P values**
Reference 1: HIV-negative individuals (n=53)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
PLWH (n=129)	-0·18 (-0·36, -0·001)	0·049	-0·80 (-1·15, -0·46)	<0·001	-0·31 (-0·51, -0·12)	0·002	-0·64 (-1·05, -0·23)	0·002
PLWH with $CD4^+$ T cell counts<500 (n=32)	-0·29 (-0·58, -0·003)	0·047	-1·31 (-1·78, -0·84)	<0·001	-0·49 (-0·75, -0·22)	<0·001	-0·82 (-1·32, -0·32)	0·002
PLWH with $CD4^+$ T cell counts≥500 (n=97)	-0·12 (-0·31, 0·07)	0·21	-0·65 (-1·01, -0·30)	<0·001	-0·26 (-0·47, -0·06)	0·01	-0·58 (-1·00, -0·17)	0·01
PLWH with detectable viral load (n=54)	-0·29 (-0·53, -0·05)	0·02	-1·15 (-1·62, -0·68)	<0·001	-0·50 (-0·77, -0·23)	<0·001	-0·75 (-1·26, -0·25)	0·004
PLWH with undetectable viral load (n=75)	-0·18 (-0·39, 0·03)	0·09	-0·71 (-1·06, -0·37)	<0·001	-0·26 (-0·45, -0·07)	0·008	-0·65 (-1·09, -0·22)	0·004
Reference 2: Fully vaccinated HIV-negative individuals (n=51)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Fully vaccinated PLWH (n=94)	-0·15 (-0·35, 0·04)	0·13	-0·68 (-1·03, -0·33)	<0·001	-0·27 (-0·48, -0·07)	0·01	-0·61 (-1·00, -0·22)	0·002

Adjusted B: adjusted correlation coefficients, adjusted for background characteristics with significant between-group difference in Table 1 (age group, gender, presence of chronic conditions other than HIV, types of SARS-CoV-2 vaccine, time interval between prime and second dose, and SARS-CoV-2 vaccination status) .

Table 5 Factors associated with SARS-CoV-2 total antibody, neutralizing antibody, S-IgG, and T cell specific immune response levels among people living with HIV (PLWH) (n=129)

	Total antibody		Neutralizing antibody		S-IgG		T cell specific immune response	
	Unadjusted B (95%CI)	**Adjusted B (95%CI)**	**Unadjusted B (95%CI)**	**Adjusted B (95%CI)**	**Unadjusted B (95%CI)**	**Adjusted B (95%CI)**	**Unadjusted B (95%CI)**	**Adjusted B (95%CI)**
Socio-demographics								
Age (years)								
18-29	Ref		Ref		Ref		Ref	
30-39	-0·06		0·06		0·03		-0·07	
	(-0·57, 0·45)		(-0·14, 0·25)		(-0·26, 0·32)		(-0·51, 0·38)	
40-49	0·17		0·08		-0·09		-0·08	
	(-0·52, 0·86)		(-0·18, 0·35)		(-0·48, 0·31)		(-0·69, 0·53)	
50-59	-0·32		-0·03		0·03		-0·56	
	(-1·51, 0·87)	---	(-0·49, 0·43)	---	(-0·66, 0·71)	---	(-1·62, 0·49)	---
Gender								
Male	Ref		Ref		Ref		Ref	
Female	1·47		0·77		0·63		1·14	
	(-1·02, 3·97)	---	(-0·18, 1·73)	---	(-0·81, 2·06)	---	(-1·07, 3·34)	---
Presence of chronic conditions other than HIV/ADIS								
No	Ref		Ref		Ref		Ref	
Yes	0·12		-0·07		-0·01		-0·20	
	(-0·42, 0·66)	---	(-0·28, 0·14)	---	(-0·30, 0·32)	---	(-0·68, 0·28)	---
Characteristics related to HIV infection								
Years since HIV diagnosis (years)								
≤1	Ref	Ref	Ref	Ref	Ref		Ref	
2-5	0·55	0·71	0·21	0·27	0·21		0·46	
	(-0·12, 1·22)	(0·23, 1·19)**	(-0·05, 0·46)	(0·05, 0·48)*	(-0·18, 0·60)		(-0·14, 1·06)	
6-10	0·82	0·49	0·33	0·23	0·33		0·56	
	(0·10, 1·53)*	(-0·03, 1·00)†	(0·05, 0·60)*	(-0·01,0·46)†	(-0·09, 0·74)		(-0·08, 1·19)†	
>10	0·59	0·44	0·22	0·15	0·23		0·55	
	(-0·20, 1·38)	(-0·13, 1·05)	(-0·08, 0·53)	(-0·11, 0·20)	(-0·23, 0·69)	---	(-0·15, 1·26)	---
Viral load (cp/ml)								
Undetectable	Ref	Ref	Ref		Ref	Ref	Ref	Ref
61-200	-0·39	-0·24	-0·17		-0·33	-0·19	-0·15	-0·01
	(-0·89, 0·10)	(-0·61, 0·14)	(-0·36, 0·03)†		(-0·61, -0·05)*	(-0·40, 0·03)†	(-0·61, 0·30)	(-0·44, 0·42)
>200	-1·10)	-0·24	-0·23		-0·68	-0·24	-0·60	-0·27
	(-1·69, -0·51)***	(-0·69, 0·22)	(-0·47, 0·001)†	---	(-1·01, -0·34)***	(-0·50, 0·03)†	(-1·14, -0·06)*	(-0·78, 0·25)
CD4+ T cell count (cells/μL)								
<500	Ref		Ref		Ref		Ref	Ref
500-1,000	0·41		0·13		0·16		0·59	0·47
	(-0·10, 0·93)		(-0·07, 0·33)		(-0·14, 0·45)		(0·14, 1·04)*	(0·05, 0·89)*
>1,000	0·61		0·14		0·24		0·48	0·40
	(-0·15, 1·36)	---	(-0·15, 0·44)	---	(-0·20, 0·68)	---	(-0·18, 1·14)	(-0·22, 1·01)
On ART								
No	Ref		Ref		Ref		Ref	
Yes	0·47		0·12		0·13		0·34	
	(-0·99, 1·93)	---	(-0·44, 0·68)	---	(-0·71, 0·97)	---	(-0·95, 1·62)	---
SARS-CoV-2 vaccination								

SARS-CoV-2 vaccination status								
Partially vaccinated	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
0-14 days after fully vaccinated	0·68 (0·11, 1·25)*	N.A.	0·27 (0·02, 0·51)*	N.A.	0·49 (0·17, 0·80)**	N.A.	0·07 (-0·55, 0·69)	0·16 (-0·46, 0·78)
15-28 days after fully vaccinated	2·11 (1·68, 2·55)***	1·00 (0·43, 1·57)**	0·72 (0·54, 0·91)***	0·30 (0·04, 0·56)*	1·26 (1·02, 1·50)***	0·53 (0·20, 0·85)**	1·08 (0·61, 1·54)***	0·99 (0·50, 1·47)***
29-56 days after fully vaccinated	1·79 (1·32, 2·27)***	1·00 (0·43, 1·57)**	0·49 (0·28, 0·69)***	0·16 (-0·10, 0·41)	1·08 (0·82, 1·35)***	0·53 (0·20, 0·85)**	0·94 (0·42, 1·45)***	0·89 (0·37, 1·40)**
57-84 days after fully vaccinated	1·36 (0·74, 1·97)***	0·85 (0·15, 1·55)*	0·38 (0·12, 0·65)**	0·14 (-0·17, 0·45)	0·75 (0·40, 1·09)***	0·26 (-0·13, 0·65)	-0·07 (-0·74, 0·59)	-0·10 (-0·76, 0·57)
>84 days after fully vaccinated	1·31 (0·20, 2·41)*	0·29 (-0·83, 1·41)	0·15 (-0·33, 0·62)	-0·31 (-0·81, 0·19)	0·69 (0·07, 1·31)*	0·07 (-0·57, 0·70)	0·40 (-0·80, 1·60)	0·20 (-1·01, 1·41)
Type of SARS-CoV-2 vaccine								
Sinopharm	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Sinovac-CoronaVac	0·71 (0·28, 1·13)**	0·26 (-0·07, 0·59)	0·23 (-0·07, 0·40)**	0·05 (-0·10, 0·20)	0·31 (0·06, 0·55)*	0·07 (-0·11, 0·25)	0·31 (-0·08, 0·69)	---
Time interval (days) between the prime and second dose								
<21 days	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
21-28 days	1·22 (0·74, 1·69)***	0·93 (0·43, 1·43)***	0·42 (0·22, 0·64)***	0·37 (0·15, 0·59)**	0·67 (0·41, 0·94)***	0·43 (0·14, 0·71)**	0·52 (-0·02, 1·06)†	
>28 days	1·28 (0·67, 1·89)***	1·15 (0·53, 1·77)***	0·39 (0·12, 0·66)**	0·36 (0·09, 0·63)**	0·70 (0·35, 1·04)***	0·53 (0·18, 0·88)**	0·62 (-0·09, 1·32)†	
Not applicable (partially vaccinated)	-0·71 (-1·22, -0·19)**	-0·03 (0·63, 0·57)	-0·19 (-0·42, 0·03)†	-0·06 (-0·32, 0·21)	-0·47 (-0·76, -0·18)**	-0·21 (-0·54, 0·13)	-0·28 (-0·87, 0·31)	---

† *P*<0·10, * *P*<0·05, ** *P*<0·01.
Adjusted B: adjusted coefficients obtained from multivariate linear regression models using all significant variables as candidates.
---: P>0·05 in univariate analysis and was not considered in multivariate analysis.

References

1 UNAIDS. UNAIDS data 2020. available at https://aids2020.unaids.org/report/ (accessed Apr 23, 2021).

2 Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. *The New England Journal of Medicine* 2011; **365**: 493-505.

3. Plettenberg A, Brockmeyer NH, Haastert B, et al. Impact of earlier HAART initiation on the immune status and clinical course of treated patients on the basis of cohort data of the German Competence Network for HIV/AIDS. *Infection* 2011; **39**: 3-12.

4. Yang X, Su B, Zhang X, Liu Y, Wu H, Zhang T. Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: Challenges of immunological non-responders. *Journal of leukocyte biology* 2020; **107**: 597-612.

5. World Health Organization. Coronavirus disease (COVID-19): COVID-19 vaccines and people living with HIV. Retrieved from: https://www.who.int/news-room/q-a-detail/coronavirus-disease-(covid-19)-covid-19-vaccines-and-people-living-with-hiv (accessed Aug 19, 2021).

6. National Health Commission of the People's Republic of China. Technical guideline on COVID-19 vaccination (1st Edition). Available at: http://www.nhc.gov.cn/xcs/yqfkdt/202103/c2febfd04fc5498f916b1be080905771.shtml. (accessed Mar 30, 2021).

7. World Health Organization. Coronavirus disease (COVID-19): Vaccines. Available at: https://www.who.int/news-room/q-a-detail/coronavirus-disease-(covid-19)-vaccines?adgroupsurvey={adgroupsurvey}&gclid=EAIaIQobChMItc-btpTY8gIVFBLnCh2CHw5rEAAYASAAEgleBfD_BwE. (accessed Aug 20, 2021).

8. United States Department of Health and Human Services. Guidance for COVID-19 and People with HIV. Retrieved from: https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/HIV_COVID_19_GL__2021.pdf (accessed Aug 19, 2021).

9. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *The New England Journal of Medicine* 2020; **383**: 2603-15.

10. Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021; **397**: 99-111.

11. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *The New England Journal of Medicine* 2021; **384**: 403-16.

12. Sadoff J, Gray G, Vandebosch A, et al. Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. *The New England Journal of Medicine* 2021; **384**: 2187-201.

13. Shinde V, Bhikha S, Hoosain Z, et al. Efficacy of NVX-CoV2373 Covid-19 Vaccine against the B.1.351 Variant. *The New England Journal of Medicine* 2021; **384**: 1899-909.
14. Hosein SR. Encouraging results from the Pfizer-BioNTech COVID-19 vaccine in HIV-positive people. Available at: https://www.catie.ca/en/catienews/2021-05-25/encouraging-results-pfizer-biontech-covid-19-vaccine-hiv-positive-people (accessed Aug 11, 2021).
15. Ruddy JA, Boyarsky BJ, Bailey JR, et al. Safety and antibody response to two-dose SARS-CoV-2 messenger RNA vaccination in persons with HIV. *AIDS* Doi:10.1097/QAD.0000000000003017.
16. Woldemeskel BA, Karaba AH, Garliss CC, et al. The BNT162b2 mRNA Vaccine Elicits Robust Humoral and Cellular Immune Responses in People Living with HIV. *Clinical infectious diseases* Doi:10.1093/cid/ciab648.
17. Frater J, Ewer KJ, Ogbe A, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 in HIV infection: a single-arm substudy of a phase 2/3 clinical trial. *The lancet HIV* 2021; **8**: e474-85.
18. Madhi S, Koen A, Fairlie L, et al. ChAdOx1 nCoV-19 (AZD1222) Vaccine in People Living With and Without HIV. *Research square*. Available at: https://www.researchsquare.com/article/rs-322470/v1 (accessed Aug 11, 2021).
19. World Health Organization. The Sinopharm COVID-19 vaccine: What you need to know. Available at: https://www.who.int/news-room/feature-stories/detail/the-sinopharm-covid-19-vaccine-what-you-need-to-know (accessed Aug 27, 2021).
20. World Health Organization. The Sinovac COVID-19 vaccine: What you need to know. Available at: https://www.who.int/news-room/feature-stories/detail/the-sinovac-covid-19-vaccine-what-you-need-to-know (accessed Aug 27, 2021).
21. The state Council the People's Republic of China. Over three billion doses of SARS-CoV-2 vaccines were manufacturerd by China in 2021. Available at: http://www.gov.cn/xinwen/2021-04/21/content_5600940.htm (accessed Aug 30, 2021).
22. Huang X, Yu M, Fu G, et al. Willingness to receive COVID-19 vaccination among people living with HIV and AIDS in China: A nationwide cross-sectional online survey. *JMIR public health and surveillance* Doi:10.2139/ssrn.3836494.
23. Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *The Lancet Infectious Diseases* 2021; **21**: 181-92.

24. National Center for AIDS/STD Control and Prevention CC. National Guideline for Detection of HIV/AIDS. Available at: http://ncaids.chinacdc.cn/zxzx/zxdteff/202005/t20200518_216798.htm (accessed Aug 5, 2021).
25. Catherine FX, Piroth L. Hepatitis B virus vaccination in HIV-infected people: A review. *Human vaccines & immunotherapeutics* 2017; **13**: 1-10.
26. Hoffman SA, Costales C, Sahoo MK, et al. SARS-CoV-2 Neutralization Resistance Mutations in Patient with HIV/AIDS, California, USA. *Emerging infectious diseases* 2021; **27**.
27. Jelicic K, Cimbro R, Nawaz F, et al. The HIV-1 envelope protein gp120 impairs B cell proliferation by inducing TGF-β1 production and FcRL4 expression. *Nature immunology* 2013; 14(12): 1256-65.
28. Okoye AA, Picker LJ. CD4(+) T-cell depletion in HIV infection: mechanisms of immunological failure. *Immunological Reviews* 2013; **254**: 54-64.
29. Cao Y, Su B, Guo X, et al. Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-Throughput Single-Cell Sequencing of Convalescent Patients' B Cells. *Cell* 2020; **182**: 73-84 e16.

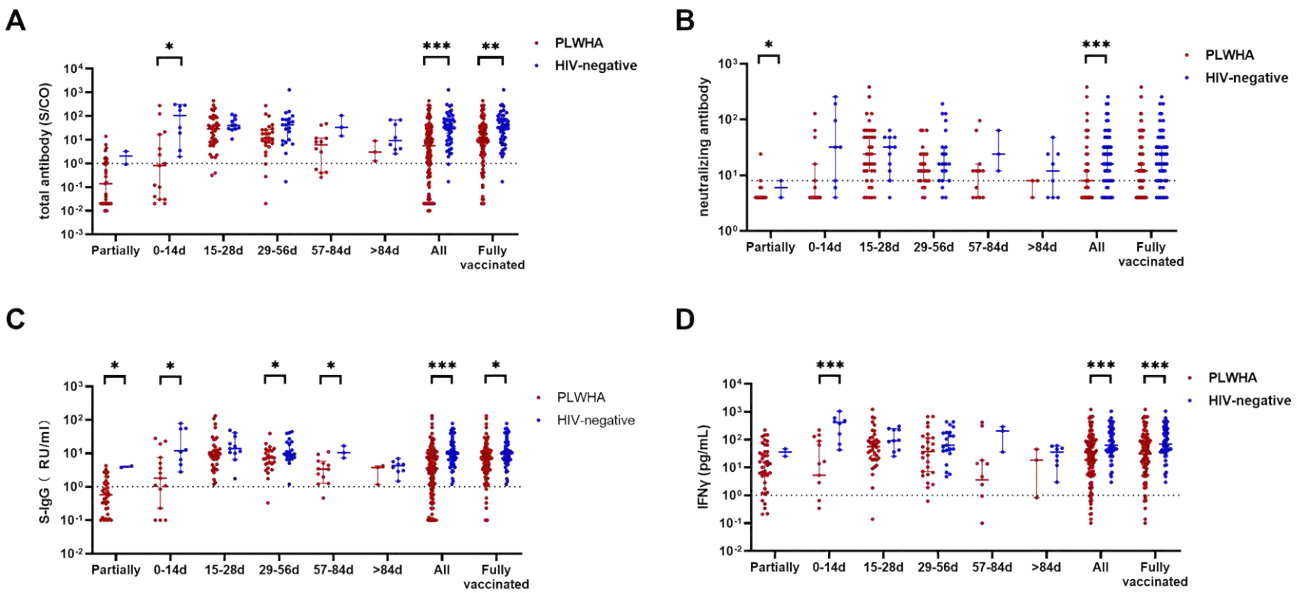


Figure 1. Levels of SARS-CoV-2 neutralizing antibody (A), total antibody (B), S-IgG (C) and T cell specific immune response (D) among HIV-negative individuals and People living with HIV (PLWH) who had received at least one dose of SARS-CoV-2 vaccine

4.10. CoronaVac induz alta resposta imune em pacientes com doença hepática gordurosa metabólica

Um artigo publicado na plataforma de preprints SSRN da revista britânica The Lancet mostrou que a CoronaVac é segura e imunogênica para indivíduos com doença hepática gordurosa associada ao metabolismo (DHGM), sendo capaz de induzir produção de anticorpos IgG em 100% dos pacientes analisados.

Participaram do estudo 50 pessoas com DHGM e 50 indivíduos saudáveis para controle, que receberam o esquema vacinal completo de duas doses da CoronaVac. A média de idade foi de 42 anos no grupo DHGM e 40 anos no grupo controle.

Um mês após a segunda dose, anticorpos IgG específicos para proteína Spike foram detectados em 100% dos indivíduos de ambos os grupos. Seis meses depois da imunização, 94% dos pacientes DHGM e 98% dos controles mantiveram a produção de anticorpos IgG. Em relação aos anticorpos neutralizantes, 82% dos pacientes e 90% dos controles apresentaram soroconversão.

O imunizante foi bem tolerado pelas pessoas com DHGM e não teve impacto no status da doença. Além disso, não houve diferença significativa na incidência geral de reações adversas entre os dois grupos e todos os efeitos relatados foram leves.

De acordo com os autores, "nosso trabalho é o primeiro estudo prospectivo de uma vacina contra Covid-19 em pacientes com DHGM publicado até o momento. Os resultados sugerem que é seguro e eficaz administrar a CoronaVac em pacientes com DHGM, e que esta vacina não afeta o estado da doença. Portanto, os pacientes com DHGM devem ser incluídos na imunização contra a SARS-CoV-2 como uma população altamente vulnerável com maior risco de morbidade e mortalidade".

A DHGM é a doença hepática mais frequente no mundo, atingindo quase 25% da população. Está associada a distúrbios metabólicos e cardiovasculares, como obesidade, resistência à insulina, hipertensão arterial, dislipidemia e diabetes tipo 2.

Publicado em: 5/10/2021

Evaluation of Immune Response and Disease Flares in metabolic-associated fatty liver disease (MAFLD) Patients Following SARS-CoV-2 Vaccination: a prospective study

Qianru Zhu[1,2*], jin Gao[3*], Jiaping Gu[4], Lu Shen[4], Jing Liu[5], Yu Song[6], Xiying Gong[6], Yutong Chen[4], Jie Liao[6], Yining He[4], Siyi Zhang[4], Lei Sun[4], Li Shao[1,4#], Jie Li[7,8#], Junping Shi[5#]

* Contributed equally

[1]Department of Translational Medicine Platform, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, Zhejiang, China;

[2]State Key Laboratory of Quality Research in Chinese Medicines, Faculty of Chinese Medicine, Macau University of Science and Technology, Macau, P.R. China;

[3]Department of Clinical Laboratory, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, Zhejiang, China;

[4]Medical college of Hangzhou Normal University, Hangzhou, Zhejiang, China;

[5]Department of Infectious & Hepatology Diseases, Metabolic Disease Center, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, Zhejiang, China;

[6]The Fourth School of Clinical Medicine, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China;

[7]Department of Infectious Diseases, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu, China;

[8]Institute of Viruses and Infectious Diseases, Nanjing University, Nanjing, Jiangsu, China

Correspondence to:

Li Shao, Department of Translational Medicine Platform, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, Zhejiang, China; Medical college of Hangzhou Normal University, Hangzhou, Zhejiang, China; Email: 20200006@hznu.edu.cn;

Jie Li, Institute of Viruses and Infectious Diseases, Nanjing University, Nanjing, Jiangsu, China; Department of Infectious Diseases, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu, China; Email: lijier@sina.com;

Junping Shi, Department of Hepatology & Infectious Disease, Metabolic Disease Center, The Affiliated Hospital of Hangzhou Normal University, Wenzhou Road, Hangzhou, Zhejiang, China; Email: 20131004@hznu.edu.cn

Word Count: 2394; Abstract: 300; Tables: 2; Figures: 4.

Abbreviations

MAFLD	Metabolic associated fatty liver disease
Nab	Neutralizing antibody
GMT	Geometric Mean Titers
BMI	Body mass index
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
GGT	γ-glutamyl transpeptidase
Alb	Albumin
STB	Total bilirubin
ALP	Alkaline phosphatase
LDL-c	Low-density lipoprotein cholesterol
HDL-c	High-density lipoprotein cholesterol
TC	Total cholesterol
TG	Triglyceride
Glu	Glucose
HOMA-IR	homeostatic model assessment of insulin resistance
hs-CRP	high-sensitive C-reactive protein
UA	Urid acids
Cr	Creatine

Author Contributions

JP Shi conceptualized and supervised the study, QR Zhu, L Shao, J Li, and JP Shi designed the study, QR Zhu, J Gao, JP Gu, L Shen, J Liu, Y Song, XY Gong, YT Chen, J Liao, YN He, SY Zhang collected data, QR Zhu, L Shao, J Li drafted the manuscript, QR Zhu, L Shao, J Li interpreted data, all authors critically reviewed or revised the manuscript and approved the final version of the manuscript.

Declaration of Interests

JP Shi reports grants from Project of Key Medical Disciplines of Hangzhou for the Department of infectious & Hepatology. QR Zhu reports grants from the Health and Science and Technology Planning Project of Hangzhou municipal Health Commission, during the conduct of the study. All authors declare no competing interests.

Research in context

Evidence before this study

In patients with metabolic-associated fatty liver disease (MAFLD), existing retrospective data on the risk of adverse outcomes with SARS-CoV-2 infection have been reported. Although the development of SARS-CoV-2 vaccines has shown encouraging safety and efficacy data in many clinical trials, However, concerns have been raised recently about SARS-CoV-2 vaccine responses in patients with MAFLD, such as safety, immunogenicity, and disease flares.

Added value of this study

To our knowledge, this is the first prospective study of the safety, immunogenicity, and disease flares of SARS-CoV-2 vaccine in MAFLD populations. We found that SARS-CoV-2 vaccination does not promote disease progression of MAFLD and metabolic comorbidities, and MAFLD patients show a robust immune response after SARS-CoV-2 vaccination in the short term, but this response does not seem to be sustained in the long term. Furthermore, NAFLD fibrosis score was a negatively predictor of neutralizing maintenance.

Implications of all the available evidence

Although previous studies reported that metabolic disorders might be significant risk factors of hospitalization and severity in COVID-19 patients, and the effectiveness of vaccination for the MAFLD population is uncertain. Our study showed that a two-dose regimen of CoronaVac vaccination in MAFLD patients was safe and well tolerated. The neutralizing antibody responses appeared to be robust in MAFLD patients who completed vaccination, which conferred 82% protection against COVID-19, and SARS-CoV-2 vaccine did not affect disease flares in MAFLD patients. Therefore, MAFLD patients should be involved in immunization to against SARS-Cov-2. However, liver fibrosis/cirrhosis maybe affect the neutralizing antibody maintenance in MAFLD patients, then future studies should consider booster doses in those with undetectable and suboptimal antibody responses.

Summary（300/300 words）

Background The ongoing COVID-19 pandemic has led to the focused application of resources toward developing vaccines to prevent COVID-19. However, the efficacy and safety profiles of vaccines against SARS-CoV-2 in patients with metabolic associated fatty liver disease (MAFLD) are still unknown. We aimed to evaluate the safety, tolerability, seroreactivity, and disease flares after SARS-CoV-2 vaccination in MAFLD patients.

Methods For this prospective observational study, we recruited patients receiving two doses SARS-CoV-2 vaccine (CoronaVac). Neutralizing antibody to the SARS-CoV-2 spike receptor-binding domain and IgG to SARS-COV-2 spike-specific were evaluated on Day 0, Day 28, Day 57, and Day 180. All participants with available data were included in the safety and immunogenicity, and disease flares analyses.

Findings 50 MAFLD patients and 50 healthy controls receiving a 0-28 interval vaccination procedure were enrolled. The seroconversion rates of neutralizing antibodies were 16% in MAFLD group (Log_{10} Geometric Mean Titers (GMT): median 0·783, IQR: 0·719-0·971) and 32% in non-MAFLD group (0·884, IQR: 0·716-1·027) on day 28, and 82% in MAFLD group (1·206, IQR: 1·053-1·467), 90% of non-MAFLD group (1·360, IQR: 1·130-1·464) on day 57, respectively. However, the neutralizing antibody titer in two groups fell below the seropositivity cut-off value on day 180 (MAFLD group 0.928, IQR: 0·773-1·057 vs. non-MAFLD group 0·907, IQR: 0·810-1·009). There was no significant difference in the overall incidence of adverse reactions after two-dose vaccinations between two groups. Furthermore. disease flares were not found in MAFLD group after two-dose vaccinations. On

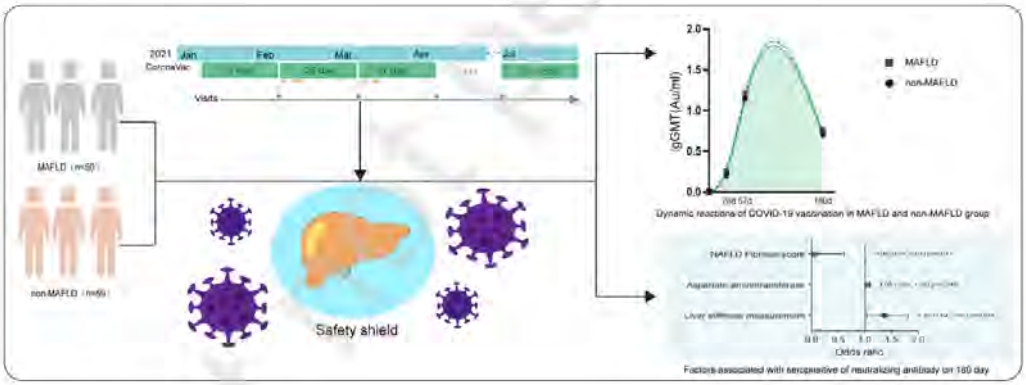
multivariable analysis, NAFLD fibrosis score was negatively associated with seropositive of neutralizing antibody on 180 days (OR 0·03, 95% CI 0·001-0·58, P = 0·022).

Interpretation Two-dose regimen of CoronaVac vaccination in MAFLD patients was safe and well tolerated. MAFLD patients showed a robust immune response after SARS-COV-2 vaccination, which conferred 82% protection against COVID-19 and vaccination does not affect MAFLD disease status.

Keywords COVID-19; SARS-COV-2 vaccination; MAFLD; safety; immunogenicity; disease flares

Funding Project of Key Medical Disciplines of Hangzhou, the Health and Science and Technology Planning Project of Hangzhou municipal Health Commission (No. A20210205).

Graphical abstract



Introduction

The persistent COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to high morbidity and mortality worldwide.[1] Metabolic-associated fatty liver disease (MAFLD), formerly named as non-alcoholic fatty liver disease (NAFLD), is the most common chronic liver disease, affecting about a quarter of the world's adult population,[2,3] which often concurrent with elements of metabolic syndromes, such as diabetes, obesity, or hyperlipidemia are

more susceptible to infection and also induce worse outcomes in COVID-19.[4,5] Early data provided evidence that metabolic syndrome was associated with chronic low-grade inflammation that compromised the immune system and caused microvascular endothelial dysfunction, which was particularly vulnerable to COVID-19 infection and disease progression.[6-8] Furthermore, COVID-19 infection is reported to associate with disease flares in MAFLD patients.[9]

Although encouraging safety and efficacy data of SARS-CoV-2 vaccines has shown in many clinical trials,[10-12] but these studies only included a small number of participants with pre-existing liver disease, such like liver transplantation.[13,14] Recently, Wang and colleagues demonstrated that COVID-19 vaccination is safe and effective in NAFLD patients, while this retrospective study did not set up the control group.[15] However, concerns have been raised recently about SARS-CoV-2 vaccine responses in MAFLD patients, such as safety, immunogenicity, and disease flares. Thus, our study aimed to examine the safety, efficacy, and changes of multiple metabolic indicators of SARS-CoV-2 vaccines in patients with MAFLD.

Methods

Study design and participants

We performed a prospective, observational cohort study that recruited adults (>18 years) receiving SARS-CoV-2 vaccination between 12 January 2021 to 4 February 2021 at the affiliated hospital of Hangzhou Normal University. All participants received two doses of an inactivated vaccine against SARS-CoV-2 (0·5 mL/dose, Sinovac life science, Beijing, China) with a 28-day interval. Hepatic steatosis was defined as a controlled attenuation parameter measurement of 248 dB/m or more.[16,17]

MAFLD diagnosed by hepatic steatosis plus any of the following three metabolic disorders according to the definition proposed by the international expert group[18]: 1) overweight/obesity (≥23 kg/m²); 2) type-2-diabetes mellitus or 3) metabolic dysregulation. Metabolic dysregulation was defined as the presence of at least two of the following metabolic risk abnormalities: 1) Waist circumference ≥ 90/80 cm in men and women; 2) Blood Pressure ≥130/85 mmHg or use of antihypertensive medications; 3) Triglyceride (TG) ≥ 150 mg/dL or use of lipid-lowing medications; 4) HDL-cholesterol (HDL-c) < 40/50 mg/dL for male and female or use of lipid-lowing medications; 5) prediabetes (fasting glucose levels 100-125 mg/dL, 2h glucose levels 140-199 mg/dL or HbA1c 5·7%-6·4%; 6) homeostatic model assessment of insulin resistance (HOMA-IR) ≥2·5.[19] We also included immunized non-MAFLD participants without hepatic steatosis, diabetes, and were of normal weight from the same hospital. The study was approved by local Hospital Ethics Committee (2021(E2)-KS-049) and written informed consent was obtained from patients involved before enrolment when data were collected. This trial had been registered in Chinese ClinicalTrials.gov (ChiCTR2100042717).

Procedures

Blood samples were captured before vaccination (Day 0), 28 days after the first vaccine dose (Day 28), 28 days after the second dose vaccination (Day 57), and 180 days after the first vaccine dose (Day 180). Telephone consultations evaluated reactogenicity and safety of each patient within 28 days after injection. Adverse events were graded according to the following scale: grade 1 (mild; does not interfere with activity); grade 2 (moderate; interferes with activity), grade 3 (severe; prevents daily activity), and grade 4 (potentially life-threatening; emergency department visit or hospital admission).

[20] Seroreactivity and biochemical indicators were detected at each time point. Neutralizing antibodies (NAb) to the receptor-binding domain (RBD) of SARS-CoV-2 spike protein was detected by iFlash 2019-nCoV NAb assay (SHENZHEN YHLO BIOTECH CO., LTD, Shenzhen, China, Cat#C86109), which is a paramagnetic particle chemiluminescent immunoassay (CLIA) for the qualitative detection of SARS-CoV-2 NAb in human serum and plasma using the automated iFlash immunoassay system, and the cut-off value of 10·00 AU/mL for the antibody.[21] IgG to SARS-COV-2 spike-specific were detected by magnetic particle chemiluminescence immunoassay using SARS-CoV-2 IgG detection kit (Beijing Hotgen Biotech Co., Ltd.). The cut-off was set as 1·00 Au/ml according to the manufacturer's guidelines.

Statistical analysis

All participants with available data were included in the safety and immunogenicity analyses. Statistics were computed in IBM SPSS Statistics 26 (Armonk, NY: IBM Corp). The significance threshold for p values was less than 0·05 after correction for multiple comparisons. We used the Pearson χ^2 test or Fisher's exact test for the analysis of categorical outcomes. We calculated Geometric Mean Titers (GMT) and corresponding IQR of the log-transformed antibody titre then used the t-test method to compare the log-transformed antibody titre. Repeated measures ANCOVA, as implemented under the mixed model,[22] was applied with change from baseline as the dependent variable, group, time, and the group by time interaction as independent variables. The approximate normality of each outcome and the change score of the outcome was confirmed by examination. Age, sex, BMI, and hypertension were included as covariates to ensure statistical balance was not captured by randomization, and reduce error

variance. Logistic regression analysis was used to investigate the association of seroconversion of Nab at day 180 with various metabolic indicators.

Role of the funding source

The funder of this study participated in study design, data collection, data analysis, and data interpretation in collaboration with all investigators.

Results

Study design and participants

A total of 164 subjects were screened in the study, and divided into MAFLD group and non-MAFLD group after matching the age. Finally, 60 people were included in the MAFLD group and 60 people in the non-MAFLD group. However, 10 MAFLD patients and 10 non-MAFLD participants did not complete vaccination. Finally, 50 subjects with MAFLD and 50 non-MAFLD subjects were enrolled in our study, respectively (Figure 1). The clinical characteristics of study participants were summarized in Table 1. The mean age of the MAFLD group was 42·10 (9·87), and 39·88(10·50) in the non-MAFLD group. MAFLD patients were more likely to have higher BMI and waist circumference, lower HDL-c and higher level of TG, as well as liver enzyme (alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA), high-sensitive C reactive protein (hs-CRP), and HOMA-IR, compared with non-MAFLD patients ($p<0·05$) (Table 1).

Safety

224 The overall incidence of adverse reactions was 19 (18%) of 100 participants within 28 days after the
225 first dose vaccination, 9 (18%) in the MAFLD group, and 10 (20%) in the non-MAFLD group, with no
226 significant difference between the two groups. All adverse reactions were mild and self-limiting.
227 Reported adverse events were graded according to China National Medical Products Administration
228 guidelines,[23] The most common symptom was injection-site pain, which was reported by5 (10%)
229 participants in the MAFLD group, 5 (10%) in the non-MAFLD group, followed by fatigue (4%),
230 dizziness (1%). Furthermore, there was still no significant difference in the overall incidence of adverse
231 events between two groups within 28 days after vaccinations, which was similar to the results
232 performed in the phase 2 trial of CoronaVac vaccine[24] (Figure 2, appendix p 2).
233
234 **Immunogenicity**
235 All individuals were assayed for anti-SARS-CoV-2 spike IgG responses and neutralizing antibodies to
236 the RBD of SARS-CoV-2 Spike Protein. At baseline, none of the participants had any detectable
237 neutralizing antibodies to live SARS-CoV-2. The seroconversion rates of neutralizing antibodies were
238 16% (8/50) in MAFLD group (Log_{10} GMT: median 0·783 [IQR: 0·719-0·971]) and 32% (16/50) in
239 non-MAFLD group (0·884 [0·716-1·027]) on 28 days after the first dose vaccination (Day 28).
240 Furthermore, seroconversion rates were 82% (41/50) in the MAFLD group (1·206 [1·053–1·467]) and
241 90% (45/50) in non-MAFLD group (1·360 [1·130-1·464] on 28 days after the second dose vaccination
242 (Day 57). However, the neutralizing antibody titer of 19 (38%) MAFLD patients (0·928 [0·773-1·057]
243 and 14 (28%) non-MAFLD participants (0·907 [0·810-1·009]) fell below the seropositivity cut-off

value on day 180. There was no significant difference in the ratio of GMT of Nab from 28 days to 57 days and 57 days to 180 days between two groups (Figure 3, appendix p 3).

The seroconversion rates of spike-specific IgG were 62% (31/50) in MAFLD group (Log_{10} GMT: median 0·159 [IQR: -0·203, 0·730], 70% (35/50) in non-MAFLD group (0·320 [-0·367, -0·899]) on 28 days after the first dose, and 100% in both MAFLD group (1·468 [1·054, 1·928]) and non-MAFLD group (1·643 [0·664, 1·911]) on 28 days after the second dose vaccination. On 180 days after vaccination, seroconversion rates were 94% in MAFLD group (0·851 [0·534, 1·181] and 98% in non-MAFLD group (0·865 [0·621, 1·187]). Then, we also found no significant difference between the two groups in the ratio of GMT of IgG from 28 days to 57 days and 57 days to 180 days, respectively (Figure 3, appendix p 3).

Changes of biochemical indicators

Overall, there was no difference between the two groups in the majority of the absolute value changes of biochemical indicators, such as ALT, AST, γ-glutamyl transpeptidase (γ-GGT), HDL- cholesterol, LDL-cholesterol, total cholesterol, triglyceride, glucose, HOMA-IR, UA, and creatinine after adjusting age, sex, BMI, and hypertension on day 28, day 57, day 180 (Figure 4, appendix p 4,5). In addition, there was also no difference in the majority of biochemical indicators, on day 28, day 57, day 180 in MAFLD patients (appendix p 6,7,8,9).

Factors associated with seropositive of neutralizing antibody on 180 days

As shown in Table 2, NAFLD fibrosis score (NFS), liver stiffness measurement, AST, HDL-c, triglyceride, and GMT of neutralizing antibody on 28 days and 57 days were significantly associated with seropositive of neutralizing antibody on 180 days by univariate analyses. Then, on multivariable analysis, the most parsimonious model that optimized prediction only included NFS, which meant that the odds of seropositive of neutralizing antibody at 180 days were higher in those who with lower NFS (OR 0·03, 95% CI 0·001, 0·58) (Table 2).

Discussion

Previous studies reported that the incidence of COVID-19 was higher in MAFLD group than in non-MAFLD group. [25,26] In addition, metabolic disorders might also be significant risk factors of hospitalization and severity in COVID-19 patients. [27,28] Therefore, it's urgently needed to explore the SARS-CoV-2 vaccine responses in MALFD patients as those patients may be uniquely susceptible to COVID-19 infection and disease progression. To the best of our knowledge, this is the first prospective report of the safety and immunogenicity of SARS-CoV-2 vaccine in MAFLD populations. Our study indicated that there was no significant difference in the overall incidence of adverse reactions after two-dose vaccinations between two groups, and SARS-CoV-2 vaccine did not affect the biochemical indicators in MAFLD patients. Furthermore, we detected that NAFLD fibrosis score was inversely associated with seropositive of neutralizing antibody on 180 days.

Similar to the general population[29], side effects related to the SARS-CoV-2 vaccine in MAFLD patients were mild and self-limiting, and the most common symptom was injection-site pain, followed by fatigue, dizziness, and diarrhea. No serious adverse events were reported in MAFLD patients. Our

results indicated that a two-dose regimen of 3 ug of inactivated CoronaVac vaccine administered 28 days apart to MAFLD patients was safe and well tolerated. Furthermore, we did not find changes of biochemical indicators, especially ALT, AST, γ-GGT after vaccinations in MAFLD patients, which means that CoronaVac vaccination might not affect the disease status and also prove the safety of SARS-CoV-2 vaccine in special population.

Vaccine immunogenicity is broadly assumed to require neutralizing antibodies, although its protection role against COVID-19 remains incompletely defined. Our results showed that two-dose CoronaVac induced neutralizing antibody and spike-specific IgG in MAFLD patients still comparable, which was consistent with previous study,[24] Similar to the study performed by Wang et al.,[29] CoronaVac elicited a high immune response in our cohort in the short term, with 82% vaccine efficacy in MAFLD group at 28 days after two-dose vaccinations. However, the GMT of Nab declined to below the positive cutoff titer after 6 months of vaccination in our cohort, which was also consistent with the results of Pan et al.'s study using the same vaccine.[30] Pan et al.'s study also found that a third dose vaccination, given at an interval of 6-8 months after the second dose could lead to a significant rebound in antibody levels, which indicating that booster vaccination may be necessary.

On multivariable logistic regression analysis, few variables were associated with seroconversion rates of neutralizing antibodies after vaccination and liver steatosis, abnormal liver function, and elevated BMI were not associated with the poor antibodies responses, which provides encouraging evidence for MAFLD patients, who should be more actively involved in SARS-CoV-2 immunization. However, NFS was inversely correlated with the seropositive of neutralizing antibody at 6 months, which implies

306 that liver fibrosis/cirrhosis could be an indicator for neutralizing antibody maintenance in MAFLD
307 patients. In fact, previous study indicated that SARS-CoV-2 infection in cirrhosis patients was
308 associated with 2.43-times mortality hazard, and the presence of cirrhosis among chronic liver disease
309 patients infected with SARS-CoV-2 were associated with 3.39-times mortality hazard.[32] In a
310 prospective study from USA, among 79 cirrhotic patients receiving two dose of mRNA vaccines or
311 single dose of Johnson & Johnson vaccine, 15 had suboptimal antibody response and 3 had
312 undetectable antibody, and cirrhosis was indicated to be associated with poor antibody response.[31]
313 Lower immune response was anticipated since humoral immunity is critical for antibody response after
314 the vaccination, but patients with cirrhosis are considered immunocompromised and the response was
315 disappointingly low, while these findings and precise mechanism merit further research.
316 Nevertheless, we acknowledge the following limitations. First, the sample size of the study is small.
317 Besides, this study does not evaluate T cell responses and the production of memory cells between the
318 two groups and data on immune persistence needs further study. Furthermore, the study lacks a
319 comparison to convalescent samples, especially in the absence of a correlate of protection, but these
320 have been taken into account in our further research.
321
322 Despite these limitations, we believe our observations are very important and meaningful. Our study is
323 the first prospective study of COVID-19 vaccine in MAFLD patients to date. It is safe and effective to
324 receive the SARS-Cov-2 vaccine in MAFLD patients, which does not affect disease status. Therefore,
325 MAFLD patients should be involved in immunization to against SARS-Cov-2 as the highly vulnerable
326 patient population with higher morbidity and mortality risk. However, immune response does not seem

to be sustained in the long term is a major concern and liver fibrosis/cirrhosis maybe affect the neutralizing antibody maintenance in MAFLD patients, then a third dose could be necessary to boost immunity. However, future studies should consider booster doses in those with undetectable and suboptimal antibody responses.

References

1. Walker P, Whittaker C, Watson O, et al. The impact of COVID-19 and strategies for mitigation and suppression in low- and middle-income countries. 2020; **369**(6502): 413-22.
2. Eslam M, Sanyal A, George J; International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. 2020; **158**(7): 1999-2014.e1.
3. Li J, Zou B, Yeo Y, et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999-2019: a systematic review and meta-analysis. 2019; **4**(5): 389-98.
4. Garg S, Kim L, Whitaker M, et al. Hospitalization Rates and Characteristics of Patients Hospitalized with Laboratory-Confirmed Coronavirus Disease 2019 - COVID-NET, 14 States, March 1-30, 2020. 2020; 69(15): 458-64.
5. Földi M, Farkas N, Kiss S, et al. Obesity is a risk factor for developing critical condition in COVID-19 patients: A systematic review and meta-analysis. 2020; 21(10): e13095.
6. Dongiovanni P, Meroni M, Longo M, Fracanzani AJErog, hepatology. MAFLD in COVID-19 patients: an insidious enemy. 2020; 14(10): 867-72.
7. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators of inflammation 2010; 2010.
8. Hegyi PJ, Váncsa S, Ocskay K, et al. Metabolic Associated Fatty Liver Disease Is Associated With an Increased Risk of Severe COVID-19: A Systematic Review With Meta-Analysis. Frontiers in medicine 2021; 8: 626425.
9. Zhou YJ, Zheng KI, Wang XB, et al. Metabolic-associated fatty liver disease is associated with severity of COVID-19. Liver international : official journal of the International Association for the Study of the Liver 2020; 40(9): 2160-3.
10. Xia S, Duan K, Zhang Y, et al. Effect of an Inactivated Vaccine Against SARS-CoV-2 on Safety and Immunogenicity Outcomes: Interim Analysis of 2 Randomized Clinical Trials. Jama 2020; 324(10): 951-60.
11. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. The New England journal of medicine 2020; 383(27): 2603-15.
12. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. The New England journal of medicine 2021; 384(5): 403-16.
13. Cornberg M, Buti M, Eberhardt CS, Grossi PA, Shouval D. EASL position paper on the use of COVID-19 vaccines in patients with chronic liver diseases, hepatobiliary cancer and liver transplant recipients. J Hepatol 2021; 74(4): 944-51.
14. Marjot T, Webb GJ, Barritt AS, et al. SARS-CoV-2 vaccination in patients with liver disease:

responding to the next big question. The lancet Gastroenterology & hepatology 2021; 6(3): 156-8.
15. Wang J, Hou Z, Liu J, et al. Safety and immunogenicity of COVID-19 vaccination in patients with non-alcoholic fatty liver disease (CHESS2101): A multicenter study. J Hepatol 2021; 75(2): 439-41.
16. Karlas T, Petroff D, Sasso M, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. Journal of Hepatology 2016; 66(5): 1022.
17. Seto WK, Hui RW, Mak LY, et al. Association Between Hepatic Steatosis, Measured by ControlledAttenuation Parameter, and Fibrosis Burden in Chronic Hepatitis B. Clinical Gastroenterology & Hepatology 2018: S1542356517311862.
18. Eslam M, Newsome PN, Anstee QM, Targher G, George J. A new definition for metabolic associated fatty liver disease: an international expert consensus statement. Journal of Hepatology 2020; 73(1).
19. Eslam M, Newsome P, Sarin S, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. Journal of hepatology 2020; 73(1): 202-9.
20. Benichou C, Danan GJTI, Science R. Guidelines for the Management of Adverse Events Occurring during Clinical Trials. 1991; 25(4): 565-71.
21. Pan Y, Jiang X, Yang L, et al. SARS-CoV-2-specific immune response in COVID-19 convalescent individuals. 2021; 6(1): 256.
22. Laird N, Ware JJB. Random-effects models for longitudinal data. 1982; 38(4): 963-74.
23. Benichou C, Danan GJTI, Science R. Guidelines for the Management of Adverse Events Occurring during Clinical Trials. 1991; 25(4): 565-71.
24. Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. The Lancet Infectious diseases 2021; 21(2): 181-92.
25. Sachdeva S, Khandait H, Kopel J, Aloysius MM, Goyal H. NAFLD and COVID-19: a Pooled Analysis. SN Comprehensive Clinical Medicine 2020; 2(9).
26. Roca-Fernández A, Dennis A, Nicholls R, Mcgonigle J, Sanyal AJ. Hepatic Steatosis, Rather Than Underlying Obesity, Increases the Risk of Infection and Hospitalization for COVID-19. Frontiers in Medicine 2021; 8.
27. Hegyi P, Váncsa S, Ocskay K, et al. Metabolic Associated Fatty Liver Disease Is Associated With an Increased Risk of Severe COVID-19: A Systematic Review With Meta-Analysis. Frontiers in medicine 2021; 8: 626425.
28. Singh A, Hussain S, Antony B. Non-alcoholic fatty liver disease and clinical outcomes in patients with COVID-19: A comprehensive systematic review and meta-analysis. Diabetes & metabolic syndrome 2021; 15(3): 813-22.
29. Wang J, Hou Z, Liu J, et al. Safety and immunogenicity of COVID-19 vaccination in patients with non-alcoholic fatty liver disease (CHESS2101): A multicenter study. Journal of hepatology 2021; 75(2): 439-41.
30. Pan H, Wu Q, Zeng G, et al. Immunogenicity and safety of a third dose, and immune persistence of CoronaVac vaccine in healthy adults aged 18-59 years: interim results from a double-blind, randomized, placebo-controlled phase 2 clinical trial. medRxiv [Preprint] 2021: 2021.07.23.21261026.

31. Thuluvath P, Robarts P, Chauhan MJJoh. Analysis of antibody responses after COVID-19 vaccination in liver transplant recipients and those with chronic liver diseases. Journal of hepatology 2021: S0168-8278(21)02002-X.
32. Ge J, Pletcher M, Lai J, JG. Outcomes of SARS-CoV-2 Infection in Patients with Chronic Liver Disease and Cirrhosis: a N3C Study. medRxiv [Preprint] 2021. 2021.06.03.21258312.
33. Aggeletopoulou I, Davoulou P, Konstantakis C, Thomopoulos K, Triantos C. Response to hepatitis B vaccination in patients with liver cirrhosis. Rev Med Virol.2017;27(6).

Figure Legends

Figure 1: Flow diagram of included participants for each analysis

Figure 2: Adverse reactions of SARS-CoV-2 vaccination in MAFLD and non-MAFLD group. Incidence of adverse reactions reported within 28 days after the first dose vaccination (A) and the second dose vaccination (B) between the two groups.

Figure 3: Serological response to SARS-CoV-2 vaccine. Antibody titres of neutralizing antibodies (A) and RBD-specific IgG (B) to live SARS-CoV-2 at different timepoints after vaccination. The horizontal line represents the threshold of specific response. Short bars represent the mean values of titres. Sample comparisons tested by Mann-Whitney U and no significant differences. Line chart represents production and regression of neutralizing antibody and spike-specific IgG (C, F). Ratio of Day 57 to Day 28 represents the production of neutralizing antibody (D) and spike-specific IgG (G) between the two groups, Ratio of Day 180 to Day57 represents the regression of neutralizing antibody (E) and spike-specific IgG (H).

Figure 4: Dynamic absolute changes of biochemical indicators at different timepoints. Dynamic absolute changes of biochemical indicators at different timepoints were shown as mean (SE). Alb, Albumin; STB, total bilirubin; hs-CRP, high-sensitive C-reactive protein; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; γ-GGT, γ-glutamyl transpeptidase; ALP, Alkaline phosphatase; Glu, glucose; HOMA-IR, Homeostasis model assessment insulin resistance; TC, total cholesterol; TG, triglyceride; HDL-c, HDL-cholesterol; LDL-c, LDL-cholesterol; UA, uric acids; Cr, creatine. * $p<0{\cdot}05$.

Table 1: Patient baseline characteristics, comorbidities by MAFLD stature

Characteristics	MAFLD group (N=50)	non-MAFLD group (N=50)	*p*
Age (years)	42·10 (9·87)	39·88 (10·50)	0·279
Sex (male/Female)	30/20	9/41	0·0001
Body mass index, BMI (kg/m^2)	21·04 (1·35)	26·84 (3·27)	**0·000**
Waist circumference (cm)	92·22 (10·29)	75·56 (6·37)	**0·000**
Controlled attenuation parameter, CAP (dB/m) [a]	300·72 (36·87)	201·32 (42·72)	**0·000**
Liver stiffness measurement, LSM (kPa)	5·66 (2·00)	4·21 (1·14)	**0·000**
Fibrosis-4 index, FIB4	-3·18 (0·03)	-3·17 (0·19)	0·714
NAFLD fibrosis score, NFS	0·91 (0·51)	0·84 (0·40)	0·459
Total bilirubin (μmol/L)	19·57 (7·66)	20·42 (6·63)	0·551
Albumin (g/L)	48·52 (2·73)	48·17 (2·18)	0·485
Alanine aminotransferase, ALT (U/L)	32·27 (24·64)	13·84 (8·35)	**0·000**
Aspartate aminotransferase, AST (U/L)	25·43 (11·51)	18·00 (4·09)	**0·000**
Alkaline phosphatase, ALP (U/L)	72·96 (18·98)	61·44 (15·50)	**0·001**
γ-glutamyl transpeptidase, γGGT (U/L)	34·78 (28·30)	18·10 (12·20)	**0·000**
LDL-cholesterol, LDL-c (mmol/L)	3·33 (0·78)	2·86 (0·65)	**0·000**
HDL-cholesterol, HDL-c (mmol/L)	1·17 (0·22)	1·50 (0·35)	**0·001**
Total cholesterol, TC (mmol/L)	5·16 (0·91)	4·72 (0·80)	**0·012**
Triglyceride, TG (mmol/L)	1·44 (0·74)	0·91 (0·37)	**0·000**
Glucose, Glu (mmol/L)	4·32 (1·64)	3·97 (0·87)	0·185
HOMA-IR	3·00 (2·9)	1·29 (0·64)	**0·000**
Creatinine (μmol/L)	64·44 (14·35)	54·76 (11·27)	**0·000**
Uric Acid, UA (μmol/L)	363·12 (101·43)	265·16 (60·60)	**0·000**
hs-CRP (mg/L)	2·05 (3·27)	0·55 (0·62)	**0·003**
Leukocyte count (10^9/L)	6·83 (1·54)	5·93 (1·22)	**0·002**
Platelets count (10^9/L)	253·22 (56·24)	245·34 (62·94)	0·511
Red blood cell count (10^9/L)	5·07 (0·51)	4·65 (0·46)	**0·000**
Lymphocytes (10^9/L)	2·33 (0·56)	1·87 (0·48)	**0·000**
hemoglobin (g/L)	150·62 (17·70)	137·64 (14·37)	**0·000**
Comorbidity, N **(%)**			
Hypertension	27 (54)	6 (12)	**0·000**
Diabetes	3 (6)	0 (0)	0·242

Results are expressed as mean (SD) / count (%), [a] represent the number of MAFLD patients diagnosed by CAP were 46, hs-CRP represents high-sensitivity C-reactive protein, HOMA-IR represents Homeostasis model assessment insulin resistance. Hypertension was defined as systolic blood pressure ⩾130 or diastolic blood pressure⩾85 mmHg.

Table 2: Factors associated with seropositive of neutralizing antibody on 180 day

Characteristics	Univariable Analysis			Multivariable Analysis		
	B	OR (95% CI)	*p*	B	OR (95% CI)	*p*
NAFLD Fibrosis score, NFS	-2·75	0·06 (0·01, 0·61)	**0·017**	-3·71	0·03 (0·001, 0·58)	**0·022**
Liver stiffness measurement, LSM (kPa)	0·31	1·37 (1·04, 1·81)	**0·028**	0·30	1·34 (0·87, 2·08)	0·185
Aspartate aminotransferase, AST (U/L)	0·05	1·05(1·00, 1·10)	**0·045**	-0·01	0·99 (0·92, 1·06)	0·718
HDL-cholesterol (mmol/L)	-1·92	0·15 (0·03, 0·71)	**0·017**	-0·78	0·46(0·04, 5·06)	0·525
Triglyceride (mmol/L)	1·16	3·18 (1·55, 6·49)	**0·002**	0·86	2·37 (0·73, 7·72)	0·152
GMT of Nab at day 57	1·78	1·02 (1·00, 1·04)	**0·018**	1·12	0·08 (0·40, 23·48)	0·279
GMT of Nab at day 28	2·79	1·13 (1·04, 1·23)	**0·005**	2·45	11·59 (0·67, 201·75)	0·093

OR, odds ratio; CI, confidence interval; GMT, Geometric Mean Titers.

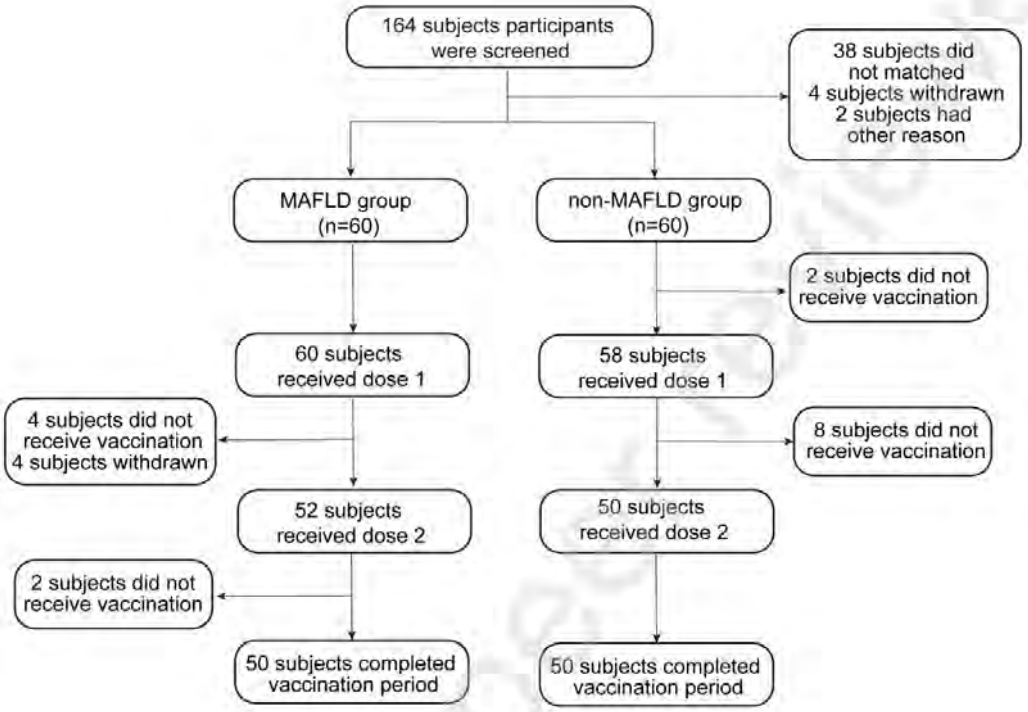


Figure 1: Screening and vaccine administration

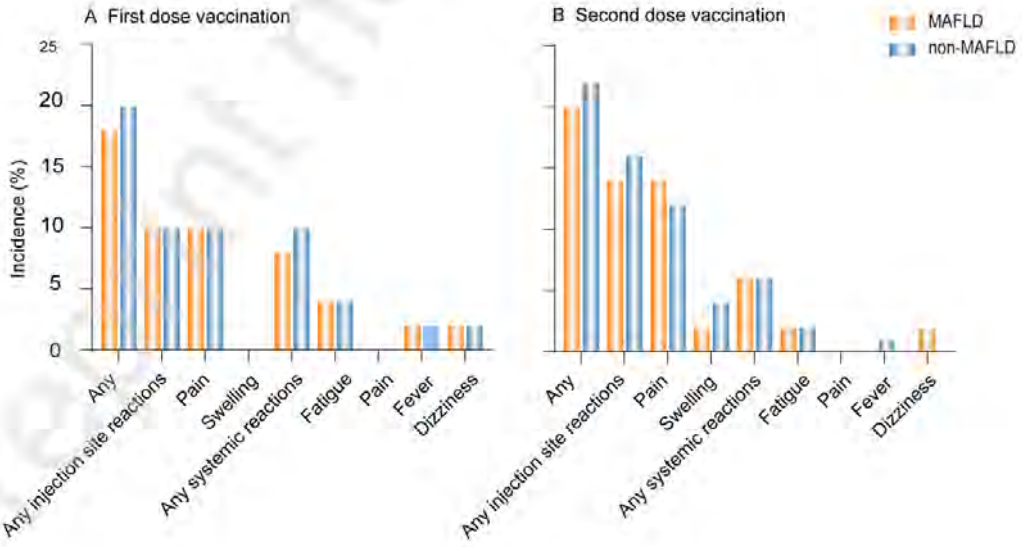


Figure 2: Adverse reactions of COVID-19 vaccination in MAFLD and non-MAFLD group

Incidence of adverse reactions reported within 28 days after the first dose vaccination (A) and the second dose vaccination (B) between the two groups.

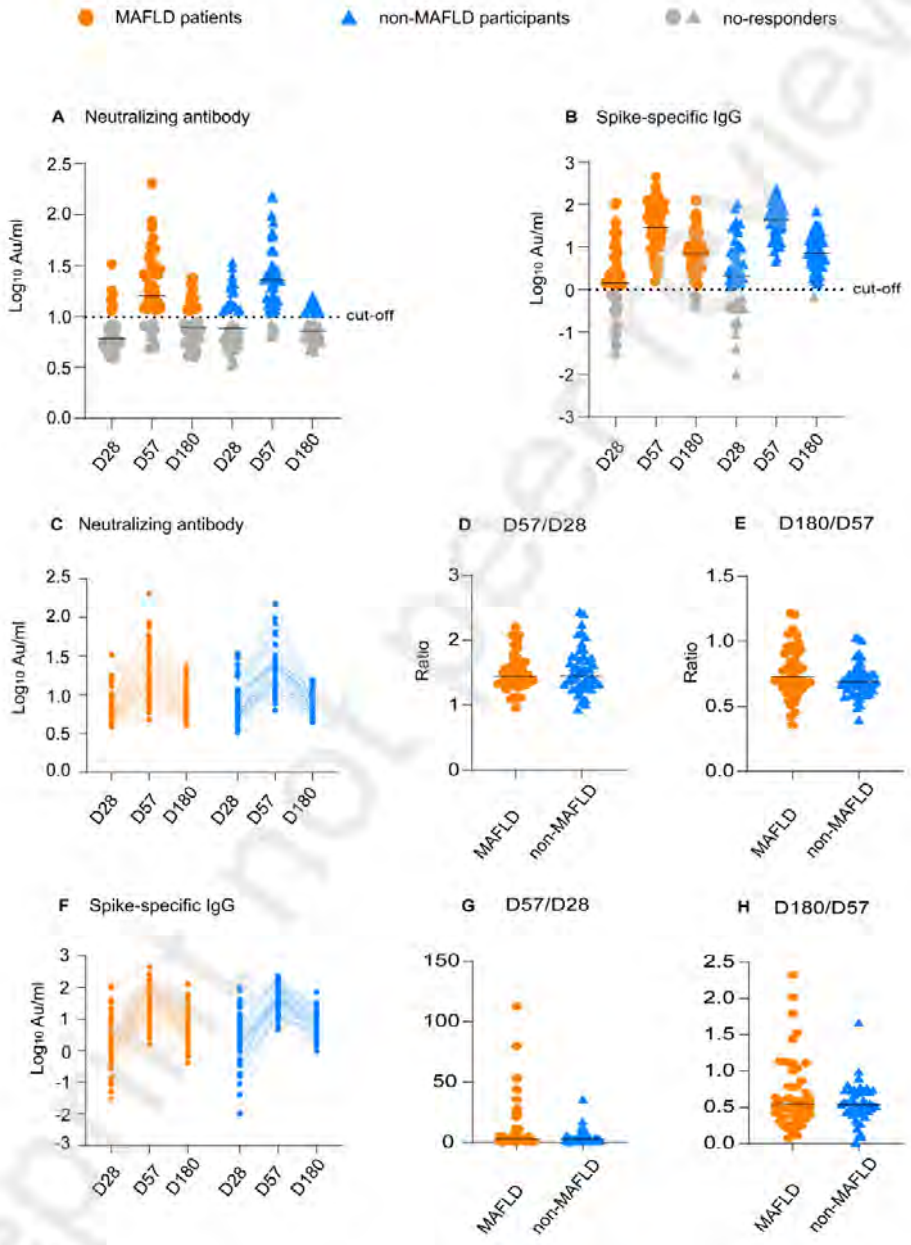


Figure 3: Serological response to COVID-19 vaccine

Antibody titres of neutralizing antibodies (A) and RBD-specific IgG (B) to live SARS-CoV-2 at different

timepoints after vaccination. The horizontal line represents the threshold of specific response. Short bars represent the mean values of titres. Sample comparisons tested by Mann-Whitney U and no significant differences. Line chart represents production and regression of neutralizing antibody and spike-specific IgG (C, F). Ratio of Day 57 to Day 28 represents the production of neutralizing antibody (D) and spike-specific IgG (G) between the two groups, Ratio of Day 180 to Day57 represents the regression of neutralizing antibody (E) and spike-specific IgG (H).

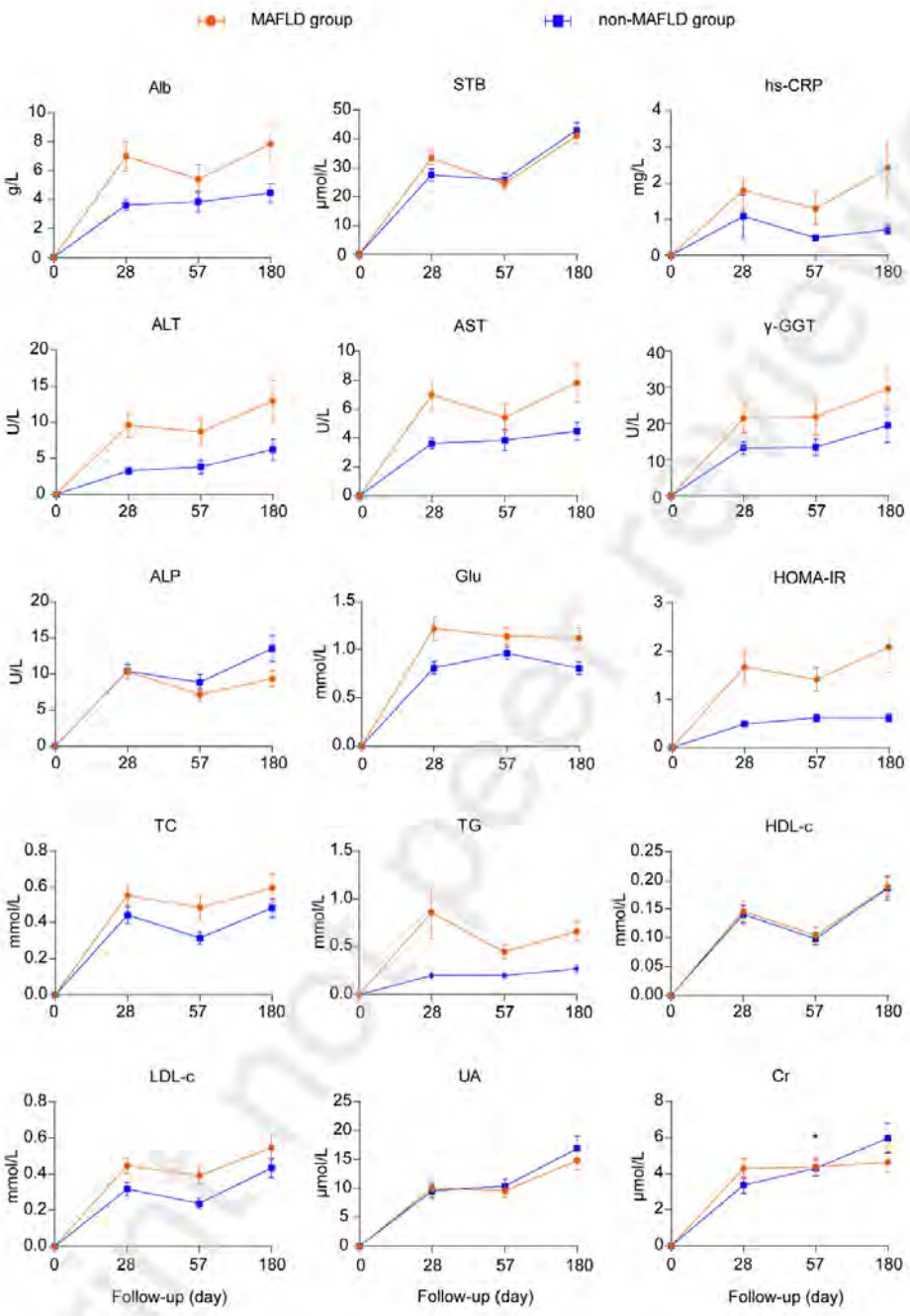


Figure 4: Dynamic absolute changes of biochemical indicators at different timepoints

Dynamic absolute changes of biochemical indicators at different timepoints were shown as mean (SE). Alb, Albumin; STB, total bilirubin; hs-CRP, high-sensitive C-reactive protein; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; γ-GGT, γ-glutamyl transpeptidase; ALP, Alkaline

phosphatase; Glu, glucose; HOMA-IR, Homeostasis model assessment insulin resistance; TC, total cholesterol; TG, triglyceride; HDL-c, HDL-cholesterol; LDL-c, LDL-cholesterol; UA, uric acids; Cr, creatine. * p<0·05.

4.11. Estudo comprova a eficácia da CoronaVac contra a Covid-19 em pacientes com câncer

Um estudo realizado na Turquia e publicado na revista Future Oncology mostrou que a CoronaVac, vacina do Butantan e da biofarmacêutica chinesa Sinovac, é eficaz e gera proteção em relação à Covid-19 em pacientes em tratamento contra o câncer. Duas semanas após a aplicação da segunda dose do imunizante, houve soroconversão (ou seja, formação de anticorpos) em 63,8% das pessoas analisadas.

A taxa de imunogenicidade chegou a 100% nos pacientes que recebem apenas anticorpo monoclonal ou imunoterapia como medicação. Além disso, nenhum dos pacientes apresentou infecção por Covid-19 em um acompanhamento médio de 85 dias após completarem o esquema vacinal. O intervalo entre a aplicação das duas doses de CoronaVac foi de 28 dias.

Este é o primeiro estudo já publicado que analisa a eficácia da CoronaVac em pacientes oncológicos. As conclusões estão no artigo Immunogenicity and safety of the CoronaVac vaccine in patients with cancer receiving active systemic therapy, escrito por pesquisadores que trabalham em sete hospitais e duas universidades de Ancara.

A pesquisa foi realizada entre janeiro e abril de 2021 com 47 pacientes com tumores sólidos. Eles tinham, em ordem de frequência, câncer colorretal, câncer de mama, de pulmão, geniturinário, gástrico, de pâncreas, ginecológico, do trato biliar e do sistema nervoso central. A maioria dos pacientes foi diagnosticada com doença em estágio IV e recebia tratamento sistêmico paliativo. A idade média dos pacientes era de 73 anos, e nenhum deles havia tido contato com o vírus SARS-CoV-2.

Além da imunogenicidade, o estudo analisou a segurança da vacina. Após receberem a primeira e a segunda dose da CoronaVac, as taxas de efeitos adversos de qualquer grau entre os 47 pacientes analisados foram de 18,9% e 23,1%, respectivamente. Não foram observados efeitos adversos graves.

Os resultados do estudo turco se somam a outros artigos divulgados recentemente que também confirmam a eficácia da CoronaVac em pessoas imunossuprimidas, um público que possui maior dificuldade na defesa imunológica do organismo.

Uma pesquisa do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) mostrou que pacientes com doenças reumatológicas autoimunes apresentaram um aumento de 70,4% no nível de anticorpos contra o vírus SARS-CoV-2 duas semanas após receberem a segunda dose da CoronaVac. Além disso, cientistas da Universidade Federal de São Paulo (Unifesp) e do Hemocentro de Ribeirão Preto da Universidade de São Paulo (USP) concluíram que 43% dos pacientes transplantados de rim analisados geraram anticorpos contra a Covid-19 15 dias após receberem a segunda dose da vacina.

Publicado em: 3/08/2021

Research Article

For reprint orders, please contact: reprints@futuremedicine.com

Immunogenicity and safety of the CoronaVac vaccine in patients with cancer receiving active systemic therapy

Cengiz Karacin*[1,2], Tulay Eren[3], Esra Zeynelgil[3], Goksen Inanc Imamoglu[3], Mustafa Altinbas[3], Ibrahim Karadag[2], Fatma Bugdayci Basal[2], Irem Bilgetekin[2], Osman Sutcuoglu[4], Ozan Yazici[4], Nuriye Ozdemir[4], Ahmet Ozet[4], Yesim Yildiz[5], Selin Akturk Esen[6], Gokhan Ucar[6], Dogan Uncu[6], Bedia Dinc[7], Musa Baris Aykan[8], İsmail Erturk[8], Nuri Karadurmus[8], Burak Civelek[9], İsmail Çelik[10], Yakup Ergun[11], Mutlu Dogan[2] & Omur Berna Oksuzoglu[2]

[1]Department of Medical Oncology, Recep Tayyip Erdogan University Training & Research Hospital, Rize, Turkey
[2]Department of Medical Oncology, HSU Dr Abdurrahman Yurtaslan Oncology Training & Research Hospital, Ankara, Turkey
[3]Department of Medical Oncology, HSU Diskapi Yildirim Beyazit Training & Research Hospital, Ankara, Turkey
[4]Department of Medical Oncology, Gazi University, Ankara, Turkey
[5]Department of Infectious Diseases & Clinical Microbiology, Gazi University, Ankara, Turkey
[6]Department of Medical Oncology, Turkish Ministry of Health Ankara City Hospital, Ankara, Turkey
[7]Department of Medical Microbiology, Turkish Ministry of Health Ankara City Hospital, Ankara, Turkey
[8]Department of Medical Oncology, HSU Gulhane Training & Research Hospital, Ankara, Turkey
[9]Department of Medical Oncology, A Life Hospital, Ankara, Turkey
[10]Department of Preventive Oncology, Institute of Oncology, Hacettepe University, Ankara, Turkey
[11]Department of Medical Oncology, Batman Training & Research Hospital, Batman, Turkey
*Author for correspondence: Tel.: +90 312 339 0000; cengizkaracin@yahoo.com

Aim: To evaluate the immunogenicity and safety of the CoronaVac vaccine in patients with cancer receiving active systemic therapy. **Methods:** This multicenter, prospective, observational study was conducted with 47 patients receiving active systemic therapy for cancer. CoronaVac was administered as two doses (3 μg/day) on days 0 and 28. Antibody level higher than 1 IU/ml was defined as 'immunogenicity.' **Results:** The immunogenicity rate was 63.8% (30/47) in the entire patient group, 59.5% (25/42) in those receiving at least one cytotoxic drug and 100% (five of five) in those receiving monoclonal antibody or immunotherapy alone. Age was an independent predictive factor for immunogenicity (odds ratio: 0.830; p = 0.043). **Conclusion:** More than half of cancer patients receiving active systemic therapy developed immunogenicity.

Tweetable abstract: Immunogenicity developed with CoronaVac in 25 (59.5%) of 42 patients who received at least one cytotoxic drug and in all patients (n = 5) who received monoclonal antibody or immunotherapy alone.

First draft submitted: 12 May 2021; Accepted for publication: 22 July 2021; Published online: 3 August 2021

Keywords: cancer • chemotherapy • COVID-19 • immunogenicity • immunotherapy • monoclonal antibody • safety • tumors • vaccine

The coronavirus disease 2019 (COVID-19) pandemic has affected millions of people worldwide and caused more than 3 million deaths [1]. Advanced age and chronic disease are major risk factors for increased COVID-19 morbidity and mortality [2]. Cancer patients constitute a particular subgroup that needs more care because of delays in diagnostic and therapeutic processes during the pandemic leading to higher mortality rates [3,4]. Vaccines developed against COVID-19 have been promising for cancer patients as well as healthy individuals [5].

CoronaVac is an inactivated COVID-19 vaccine that has been shown to have immunogenicity, with vaccine-induced neutralizing antibodies to SARS coronavirus 2 (SARS-CoV-2) that can neutralize ten representative strains

Future Medicine

of SARS-CoV-2 [6,7]. In a phase II study, a highly automated bioreactor (ReadyToProcess WAVE 25 rocker; Cytiva, Umeå, Sweden) was used to produce the vaccine. Immunogenicity is provided by the high content of intact spike proteins in the vaccine. It has been used in many countries, including China and Turkey. The CoronaVac vaccine was approved by World Health Organization (WHO) after results of the phase III trial's interim analysis [8].

Experiences from influenza vaccine trials have given rise to thinking about possible lower immunogenicity rates in patients who are on active immunosuppressive therapy [9,10]. However, seasonal influenza vaccines have a protective effect even in cancer patients who receive active systemic treatment, although they develop less immunogenicity than healthy people [9]. In COVID-19 vaccine trials, receiving immunosuppressive therapy was an exclusion criterion, so patients on immunosuppressants (including cancer patients) were not included in the trials [6,7]. This therefore obscures the effectiveness of the COVID-19 vaccine in patients with a cancer diagnosis. Although there are no randomized controlled clinical trial data evaluating the immunogenicity of the COVID-19 vaccine in cancer patients who are on active systemic therapy, the COVID-19 vaccine is recommended for these patients by leading and local guidelines [11,12]. This multicenter, prospective, observational study aimed to evaluate the immunogenicity and safety of the CoronaVac vaccine in patients with solid organ tumors receiving active systemic therapy (cytotoxic chemotherapy, monoclonal antibody, immunotherapy).

Methods

This multicenter, prospective, observational study was conducted with patients diagnosed with solid organ tumors receiving active systemic therapy. Ethics committee approval (2021-01/963) and Ministry of Health permission for the study were obtained on January 13, 2021. An informed consent form was obtained from all patients included in the study. Patients who had a solid organ tumor diagnosis, active systemic therapy (cytotoxic chemotherapy, monoclonal antibody, immunotherapy), Eastern Cooperative Oncology Group performance status 0–2, life expectancy >12 weeks, age >18 years and negative SARS-CoV-2 antibody serology before the first vaccine dose were included in the study. Those who had previous COVID-19 infection, contact with COVID-19-infected people in the last 14 days or any other immunosuppressive disease (i.e., HIV infection, solid organ transplant) were excluded from the study.

Evaluation of vaccine immunogenicity was the primary outcome of the study. Secondary outcomes were determining side effects, safety and factors affecting vaccine immunogenicity (e.g., age, sex, systemic treatment regimen). Baseline blood samples to measure SARS-CoV2 antibody level were taken 0–3 days before administration of the first dose of the vaccine. There was no intervention in planned systemic treatment schedules. A second dose of the vaccine was administered 4 weeks after the first dose. Side effects were recorded after the first and second doses. A second blood sample was taken to measure antibody level 4 weeks after the last dose of the vaccine. All patients were vaccinated within the Ministry of Health's vaccination program.

Vaccine procedure

CoronaVac is an inactivated vaccine against COVID-19. The vaccine (3 µg in 0.5 ml of aluminum hydroxide diluent per dose in ready-to-use syringes) was administered intramuscularly according to a dosing schedule of day 0 and day 28. Since the study was noninterventional, a specific day was not determined between the patients' systemic treatment and administration of the vaccine by investigators. The median interval between the first dose of the vaccine and start of the previous chemotherapy cycle was 7 days (interquartile range: 5–10 days). The median interval between the second dose of the vaccine and start of the previous chemotherapy cycle was 7 days (interquartile range: 5–8 days).

Interpretation of antibody results & assessment of immunogenicity

SARS-COV-2 antibody was evaluated by Siemens Healthcare Diagnostics (Tarrytown, NY, USA) Atellica IM SARS-CoV-2 total ELISA kits approved by the US FDA. The system reports Atellica IM SARS-CoV-2 total assay results in index values and as nonreactive (<1 index) or reactive (≥1.0 index) [13]. Seroconversion (immunogenicity) was defined as post-vaccination positivity of SARS-COV-2 antibody (≥1 IU) that was negative (<1 IU) before vaccination. The antibody meter ranged from 0.05 to 10 IU, and values higher than 10 IU were reported as >10 IU. According to serum antibody level, immunogenicity was classified as low (1–5 IU), intermediate (6–10 IU), or high (>10 IU).

Statistical analysis

In the descriptive statistics of the study, numerical data were given as median (range or interquartile range) and categorical data as frequency (percentage). The Mann–Whitney U test was used to compare the continuous variables of the two independent groups. Pearson's chi-square or Fisher's exact test was used to compare categorical data. Variables with a $p < 0.20$ as a result of univariate analysis were included in the logistic regression analysis to determine the factors affecting immunogenicity. Statistical analysis was performed with SPSS Statistics 25.0 (IBM Corporation, NY, USA) for Windows (Microsoft Corporation, WA, USA), and a two-tailed $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 47 patients with solid tumors were enrolled consecutively between 25 January 2021, and 26 April 2021. The median patient age was 73 years (range: 64–80), and 61.7% were male. Primary cancer sites, in order of frequency, were colorectal, breast, lung, genitourinary, gastric, pancreas, gynecological, biliary tract, and CNS. The majority of patients were diagnosed with stage IV disease and received palliative systemic treatment. There were 42 (89.4%) patients receiving at least one cytotoxic drug, three (6.4%) receiving monoclonal antibody alone and two (4.2%) receiving immunotherapy alone. Granulocyte colony-stimulating factor was administered to 36.2% of the patients (Tables 1 & 2).

Immunogenicity

Of the 47 patients, 30 (63.8%) had seroconversion (immunogenicity). Immunogenicity developed in all five patients who received monoclonal antibody (n = 3) or immunotherapy (n = 2) alone. Immunogenicity also developed in 25 (59.5%) of 42 patients who received at least one cytotoxic drug. Antibody levels in all patients who received monoclonal antibodies were found to be higher (>10 IU) and were slightly elevated (1–5 IU) in two patients who received immunotherapy alone. Of the 25 patients who received at least one systemic cytotoxic treatment and developed immunogenicity, high (>10 IU) antibody levels were measured in four, moderate (6–10 IU) levels were measured in six and low (1–5 IU) levels were measured in 15. Detailed patient demographics, clinical characteristics and antibody levels are shown in Table 3.

In univariate analysis, patients who had immunogenicity were younger, with a median age of 72 years ($p = 0.031$), whereas the median age of those who had no seroconversion was 75 years. The immunogenicity rate was lower in those who used granulocyte colony-stimulating factor (47.1% vs. 73.3%; $p = 0.072$). There was no relationship between immunogenicity and other demographic and clinical characteristics (Table 3).

Age was defined as a significant independent predictive factor for CoronaVac immunogenicity in multivariate analysis (odds ratio: 0.830; 95% CI: 0.693–0.994; $p = 0.043$) (Table 4). None of the patients had COVID-19 infection at a median follow-up of 85 days (range: 62–98 days).

Safety analysis

Local and systemic reactions after the first and second doses of the vaccine are shown in Table 5. After the first and second doses, side effect rates of any grade were 18.9 and 23.1%, respectively. With regard to local reactions, pain at the injection site was the most common side effect; among systemic side effects, fatigue was the most common. There were no serious (grade 3 or 4) side effects or toxic deaths.

Discussion

In this study, the authors prospectively evaluated the immunogenicity and safety of the CoronaVac vaccine in patients with solid organ tumors receiving active systemic therapy. The immunogenicity rate was 63.8% for the whole patient population and 59.5% for the patients who received at least one cytotoxic chemotherapy. The phase I and II CoronaVac trial, which evaluated the immunogenicity of the CoronaVac vaccine in healthy 18- to 59-year-old individuals, had four cohorts, and 3 and 6 μg of the vaccine was administered on a schedule of 0–14 and 0–28 days [6]. However, in the authors' study, the vaccine was administered on days 0 and 28 at a dose of 3 μg. In the phase I and II CoronaVac trial, the immunogenicity rates were 95.0 and 96.5% for doses of 3 and 6 μg (days 0 and 28), respectively. Another phase I and II trial evaluated the immunogenicity and safety of the CoronaVac vaccine in a healthy elderly population (≥60 years) [7], and the immunogenicity rates were 98.0 and 99.0% in the 3 and 6-μg dose subgroups, respectively. In the present study, the immunogenicity rates with 3 μg

Table 1. Demographic and clinical features of the patients.

Demographic and clinical features	Patients (n = 47)
Age (years), median (range)	73 (64–80)
Sex, n (%)	
Male	29 (61.7)
Female	18 (38.3)
Primary malignancy, n (%)	
Colorectal	13 (27.7)
Breast	7 (14.9)
Lung	6 (12.8)
Genitourinary	6 (12.8)
Gastric	5 (10.6)
Pancreas	4 (8.5)
Gynecological	3 (6.4)
Biliary tract	2 (4.2)
CNS	1 (2.1)
TNM stage, n (%)	
II	4 (8.5)
III	10 (21.3)
IV	33 (70.2)
Treatment modality, n (%)	
Neoadjuvant	1 (2.1)
Adjuvant	15 (31.9)
Palliative	31 (66.0)
Type of anticancer treatment, n (%)	
Receiving at least one cytotoxic drug	42 (89.4)
Receiving only monoclonal antibody	3 (6.4)
Receiving only immunotherapy	2 (4.2)
Treatment group, n (%)	
3W	10 (21.3)
2W	22 (46.8)
1W	7 (14.9)
C	6 (12.8)
IO	2 (4.2)
G-CSF, n (%)	
No	30 (63.8)
Yes	17 (36.2)

1W: Cytotoxic drug or monoclonal antibody given each week; 2W: Cytotoxic drug or monoclonal antibody given every 2 weeks; 3W: Cytotoxic drug or monoclonal antibody given every 3 weeks; C: Cytotoxic drug given continuously orally; IO: Immunotherapy given every 2 weeks; TNM: Tumor, node, metastasis.

(days 0 and 28) were lower than those seen in these phase I and II CoronaVac trials. However, this study included cancer patients who were undergoing active systemic cancer treatment with chemotherapy, monoclonal antibody or immunotherapy. Although the immunogenicity rate was relatively lower in cancer patients, none had COVID-19 over a median follow-up period of 85 days.

To the authors' knowledge, this is the first study to evaluate the immunogenicity of the CoronaVac vaccine in cancer patients receiving active systemic therapy. The low immunogenicity demonstrated in the authors' study was consistent with other studies [14–17]. In a study conducted in Turkey, it was shown that patients using immunomodulators for rheumatological disease developed less immunogenicity compared with healthy individuals receiving the CoronaVac vaccine [14]. Similar results have been found in cancer patients who received the mRNA-1273 (Moderna, MA, USA) or BNT162b2 mRNA (Pfizer, NY, USA) COVID-19 vaccines [15–17]. The immunogenicity rate was found to be 53.7% in patients with hematological malignancies, of which approximately 45% received active

Table 2. Details of patient demographics, clinical features, treatment schedules and immunogenicity results.

Group	Age (years)	Sex	ECOG PS	Comorbidity	Primary	Stage	Regimen	G-CSF	Antibody IU/ml	Seroconversion
3W	64	F	1	DM, HT	Breast	III	Trastuzumab	N	>10	Y
3W	72	F	1	HT	Breast	IV	Trastuzumab	N	>10	Y
3W	74	F	0	DM, HT	Breast	III	Doxorubicin + cyclophosphamide	N	6.82	Y
3W	65	F	1	DM, HT	Breast	IV	Pertuzumab + trastuzumab	N	>10	Y
3W	65	F	1	HT, COPD	Lung	II	Etoposide + cisplatin	N	2.87	Y
3W	70	M	2	CHF	Lung	IV	Paclitaxel + carboplatin	N	>10	Y
3W	75	M	2	–	Lung	III	Paclitaxel + carboplatin	Y	0.27	N
3W	74	M	0	–	Prostate	IV	Docataxel	Y	0.87	N
3W	74	M	1	HT, CAD	Prostate	IV	Docetaxel	Y	0.64	N
3W	74	M	1	–	Gastric	IV	Docetaxel + cisplatin + 5-FU	Y	0.59	N
2W	80	M	1	–	Gastric	IV	FOLFIRI	Y	1.12	Y
2W	71	M	0	HT, CAD	Colon	IV	FOLFIRI + cetuximab	N	6.82	Y
2W	75	F	1	HT, DM	GBM	IV	Irinotecan + bevacizumab	N	>10	Y
2W	80	F	1	HT	Bladder	IV	Paclitaxel + carboplatin	Y	0.90	N
2W	73	M	1	DM	Colon	IV	FUFA + bevacizumab	N	1.58	Y
2W	69	M	0	–	Pancreas	IV	Gemcitabine 1–8	N	0.98	N
2W	80	F	1	HT	Colon	IV	FUFA + bevacizumab	N	5.29	Y
2W	71	M	1	DM, HT, COPD	Pancreas	IV	mFOLFIRINOX	Y	1.20	Y
2W	73	F	1	HT	Colon	IV	FOLFIRI	N	2.78	Y
2W	71	M	1	HT, DM	Colon	III	FUFA	N	6.31	Y
2W	72	M	1	Arrhythmia	Colon	IV	FOLFIRI + cetuximab	Y	9.15	Y
2W	78	M	1	Asthma	Pancreas	III	Gemcitabine	Y	1.66	Y
2W	74	M	1	HT, COPD	Gastric	III	FUFA	N	4.86	Y
2W	75	M	1	CAD	Colon	IV	FOLFIRI	Y	0.76	N
2W	72	F	1	–	Breast	IV	Gemcitabine	Y	0.98	Y
2W	72	M	0	HT	Bladder	IV	Gemcitabine + carboplatin	N	2.66	Y
2W	78	F	2	HT, DM	Endometrium	IV	Paclitaxel + carboplatin	N	0.86	N
2W	77	F	1	HT, COPD	Ovarian	IV	Gemcitabine	Y	0.05	N
2W	68	M	1	HT	Gastric	III	FLOT4	Y	1.05	Y
2W	65	M	1	HT, CAH	Rectum	IV	FOLFOX	N	4.42	Y
2W	77	F	2	HT, DM	Pancreas	IV	FOLFIRI	Y	>10	Y
2W	76	M	1	HT, DM, CAD	Biliary tract	IV	Gemcitabine + cisplatin	N	0.83	N
1W	73	M	1	–	Lung	IV	Paclitaxel	Y	1.05	Y
1W	77	M	1	CAH	Lung	IV	Irinotecan	N	0.19	N
1W	80	F	1	HT, DM, arrhythmia	Breast	III	Paclitaxel	Y	0.45	N
1W	66	F	0	–	Breast	II	Paclitaxel	N	0.97	N
1W	67	M	0	–	Rectum	IV	5-FU	N	>10	Y
1W	77	F	1	HT	Ovarian	IV	Paclitaxel + carboplatin	Y	7.20	Y
1W	70	M	0	–	Lung	III	Carboplatin	N	1.07	Y
C	73	F	1	–	Biliary tract	IV	Capecitabine	N	1.03	Y
C	73	M	1	Asthma	Colon	II	Capecitabine	N	1.59	Y
C	72	M	1	DM	Colon	II	XELOX	N	4.42	Y
C	73	M	2	–	Gastric	III	XELOX	N	0.80	Y
C	71	F	2	HT, DM	Rectum	IV	Capecitabine + cetuximab	N	0.05	N
C	76	M	1	–	Colon	IV	Capecitabine	N	0.95	N
IO	71	M	0	–	RCC	IV	Nivolumab	N	2.06	Y
IO	76	M	1	–	RCC	IV	Nivolumab	N	1.93	Y

1W: Cytotoxic drug or monoclonal antibody given each week; 2W: Cytotoxic drug or monoclonal antibody given every 2 weeks; 3W: Cytotoxic drug or monoclonal antibody given every 3 weeks; 5-FU: Fluorouracil; C: Cytotoxic drug given continuously orally; CAD: Coronary artery disease; CAH: Congenital adrenal hyperplasia; CHF: Congestive heart failure; COPD: Chronic obstructive pulmonary disease; DM: Diabetes mellitus; ECOG PS: Eastern Cooperative Oncology Group performance status; F: Female; FOLFIRI: Folinic acid, fluorouracil and irinotecan; FOLFOX: Folinic acid, fluorouracil and oxaliplatin; FUFA: Fluorouracil and folinic acid; FLOT4: fluorouracil plus leucovorin, oxaliplatin, and docetaxel; GBM: Glioblastoma multiforme; G-CSF: Granulocyte colony-stimulating factor; HT: Hypertension; IO: Immunotherapy given every 2 weeks; M: Male; mFOLFIRINOX: Modified folinic acid, fluorouracil, irinotecan and oxaliplatin; N: No; RCC: Renal cell carcinoma; TNM: Tumor, node, metastasis; XELOX: Capecitabine and oxaliplatin; Y: Yes.

Table 3. Univariate analysis of serological response rate.

	Seroconversion		p-value
	No	Yes	
Age (years), median (IQR)	75 (73–77)	72 (70–74)	0.031
Sex, n (%)			
Male	10 (34.5)	19 (65.5)	0.760
Female	7 (38.9)	11 (61.1)	
ECOG PS, n (%)			
0	3 (33.3)	6 (66.7)	0.249
1	10 (31.3)	22 (68.8)	
2	4 (66.7)	2 (33.3)	
Comorbidity, n (%)			
No	7 (50.0)	7 (50.0)	0.199
Yes	10 (30.3)	23 (69.7)	
TNM stage, n (%)			
II	1 (25.0)	3 (75.0)	0.767
III	3 (30.0)	7 (70.0)	
IV	13 (39.4)	20 (60.6)	
Treatment, n (%)			
Palliative	13 (41.9)	13 (58.1)	0.252
Other	4 (25.0)	12 (75.0)	
Treatment group, n (%)			
1W	3 (42.9)	4 (57.1)	NA
2W	7 (31.8)	15 (68.2)	
3W	4 (57.1)	3 (42.9)	
C	3 (50.0)	3 (50.0)	
IO	0 (0)	2 (100)	
Monoclonal AB only	0 (0)	3 (100)	
G-CSF, n (%)			
No	8 (26.7)	22 (73.3)	0.072
Yes	9 (52.9)	8 (47.1)	

1W: Cytotoxic drug or monoclonal antibody given each week; 2W: Cytotoxic drug or monoclonal antibody given every 2 weeks; 3W: Cytotoxic drug or monoclonal antibody given every 3 weeks; AB: Antibody; C: Cytotoxic drug given continuously orally; ECOG PS: Eastern Cooperative Oncology Group performance status; G-CSF: Granulocyte colony-stimulating factor; IO: Immunotherapy given every 2 weeks; IQR: Interquartile range; NA: Not applicable; TNM: Tumor, node, metastasis.

Table 4. Multivariate analysis of serological response.

	OR	95% CI	p-value
Comorbidity	2.937	0.729–11.833	0.130
G-CSF	0.468	0.116–1.881	0.284
Age	0.830	0.693–0.994	0.043

G-CSF: Granulocyte colony-stimulating factor; OR: Odds ratio.

systemic therapy [15]. In the same study, it was stated that immunogenicity decreased independently of treatment in patients with chronic lymphocytic leukemia. In another study evaluating 167 patients with chronic lymphocytic leukemia, the immunogenicity rate was found to be 39.5% with the BNT162b2 mRNA COVID-19 vaccine [16]. In a study by Massarweh *et al.* that included patients with solid organ tumors or hematological malignancies receiving active systemic therapy, it was shown that the mean antibody level detected after vaccination (BNT162b2 mRNA) was lower than that seen in healthy individuals [17].

In previous influenza vaccine studies, it has been shown that the immunogenicity rate may be lower in immunosuppressive patients compared with healthy individuals [9]. Adjuvant and high-dose vaccines are beneficial for increasing immunogenicity in seasonal influenza vaccines in immunosuppressive patients. It was also shown in a meta-analysis that the immunogenicity of the influenza vaccine was lower in cancer patients, who constituted

Table 5. Local and systemic reactions after first and second vaccine doses.

	First dose			Second dose		
	Any grade	Grade 1	Grade 2	Any grade	Grade 1	Grade 2
Total, n (%)	9 (18.9)	7 (14.7)	2 (4.2)	11 (23.1)	8 (16.8)	3 (6.3)
Local reaction, n (%)						
Pain at injection site	2 (4.2)	2 (4.2)	0 (0)	3 (6.3)	3 (6.3)	0 (0)
Swelling	1 (2.1)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)
Itchiness	1 (2.1)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)
Erythema	0 (0)	0 (0)	0 (0)	2 (4.2)	0 (0)	2 (4.2)
Systemic reaction, n (%)						
Fever	1 (2.1)	1 (2.1)	0 (0)	1 (2.1)	1 (2.1)	0 (0)
Myalgia	1 (2.1)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)
Fatigue	2 (4.2)	0	2 (4.2)	5 (10.5)	4 (8.4)	1 (2.1)
Headache	1 (2.1)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)

the immunosuppressive group, compared with healthy individuals [9,18]. In the VACANSE study in which the immunogenicity of the H1N1v vaccine was evaluated in patients with solid organ tumors receiving active systemic treatment, it was reported that a single dose of the vaccine did not provide sufficient immunogenicity [10]. However, the immunogenicity might have increased had the vaccine been administered in two doses. Similarly, the fact that immunogenicity was lower in the authors' study compared with studies using healthy individuals raised the question of whether administration of a booster CoronaVac vaccine dose may increase the immunogenicity rates; this needs further clinical trials.

With aging, many molecular changes – called immunosenescence – occur in the immune system [19]. This dysregulation in the elderly immune system causes a decrease in the immune response obtained with vaccines. Considering that advanced age is a significant risk factor for COVID-19 morbidity and mortality, elderly patients have been given priority for vaccination against COVID-19 in many countries, including the authors' [20]. One of the concerns in the vaccination of elderly patients is immunogenicity sufficiency. The CoronaVac phase I and II trial, which was conducted with elderly volunteers, showed that the vaccine developed an immunogenicity profile comparable to that seen with young adults, without any serious adverse events [7]. The authors' study showed that the only independent factor affecting immunogenicity in multivariate analysis was age ($p = 0.043$). As mentioned, immunogenicity decreases with increasing age. This point might have also contributed to the lower immunogenicity rate seen with the CoronaVac vaccine in the authors' elderly cancer patients on active cancer treatment.

In the authors' study, the cumulative rate of possible vaccine-related side effects observed after two doses of the CoronaVac vaccine was 32%. Toxicity rates were reported to be 33 and 20% in the 3-μg cohorts of the Phase I and II CoronaVac trials, which were conducted with younger and elderly healthy volunteers, respectively [6,7]. The fatigue rate in the authors' study was higher than that seen in other CoronaVac trials (14.7 vs <10 and 3%). The higher fatigue rate in the authors' patients might have been related to cancer diagnosis and its active treatment during vaccination. Similar to the CoronaVac Phase I and II trials, no serious vaccine-related adverse events were observed in the authors' study.

Some researchers have hypothesized that the vaccine could hypothetically lead to an exaggerated immune response in immunotherapy recipients [21]. However, in a study evaluating short-term safety in 134 patients who received immunotherapy and the BNT162b2 mRNA COVID-19 vaccine, it was reported that there was no increase in immunotherapy-related immune side effects [22]. In the authors' study, only two patients received imunotherapy, and they did not experience any side effects. The median interval between the vaccine and the start of the previous immunotherapy cycle was 7 days in both patients.

This study did not have a validation cohort, which was a strong limitation. The study population also consisted of elderly patients, which was another limitation. Lower immunogenicity rate in the geriatric population irrespective of vaccination is a well-known finding, so it should be kept in mind that the study results do not reflect immunogenicity with vaccination in young cancer patients receiving active systemic therapy. It is a fact that the development of immunogenicity alone does not mean absolute protection from COVID-19 infection. Despite a median follow-up period of 85 days, the authors note that this is not long enough to comment on whether the vaccine has a long-term protective effect against COVID-19 infection. Another limitation was that cellular immunity, which has a

preventive effect against COVID-19 infection, was not evaluated in this study. Comorbidities and active cancer treatment modalities might be confounding factors in the evaluation of 'real' vaccine-related side effects. Therefore, it has been stated that the side effects were 'probably' related to the vaccine. The low number of patients and absence of a control group are another limitation of the study. Despite these limitations, to the best of the authors' knowledge, this study was the first to evaluate the efficacy and safety of the CoronaVac vaccine in cancer patients undergoing active systemic cancer treatment with chemotherapy, monoclonal antibody or immunotherapy.

Conclusion

Immunogenicity developed with two doses of the CoronaVac vaccine (3 μg/day days 0 and 28) in more than half of the patients with solid organ tumors undergoing active systemic cytotoxic chemotherapy.

Future Perspective

The fact that vaccination rates do not reach the targeted levels worldwide and virus mutations show that our fight against COVID-19 will continue in the coming years. There is a need for studies investigating more effective vaccination programs in cancer patients receiving active systemic therapy.

Summary points

- This prospective observational multicenter study was conducted with 47 patients with solid organ tumors receiving active systemic therapy to evaluate the immunogenicity and safety of the CoronaVac vaccine in patients with solid organ tumors receiving active systemic therapy (cytotoxic chemotherapy, monoclonal antibody, immunotherapy).
- Evaluation of vaccine immunogenicity was the primary outcome of the study; the secondary outcome was determining the vaccine's safety.
- The median patient age was 73 (range: 64–80), and 61.7% were male. Immunogenicity developed in 25 (59.5%) of 42 patients who received at least one cytotoxic drug and in all patients (n = 5) who received monoclonal antibody or immunotherapy alone.
- In univariate analysis, patients who had immunogenicity were younger, with a median age of 72 years (p = 0.031), whereas the median age of those who had no seroconversion was 75 years.
- Immunogenicity developed in 47.1% of those who were administered granulocyte colony-stimulating factor and 73.3% of those who were not administered granulocyte colony-stimulating factor (p = 0.072).
- In multivariate analysis, the only independent predictive factor affecting immunogenicity was patient age (odds ratio: 0.830; 95% CI: 0.693–0.994; p = 0.043).
- After the first and second doses of the vaccine, side effect rates of any grade were 18.9 and 23.1%, respectively, and there were no serious (grade 3 or 4) side effects or toxic deaths.
- Immunogenicity developed with two doses of the CoronaVac vaccine (3 μg/day days 0 and 28) in more than half of the patients with solid organ tumors undergoing active systemic cytotoxic chemotherapy.

Author contributions

C Karacin contributed to study concept, study design, data analysis and interpretation and manuscript writing. T Eren, E Zeynelgil, G I Imamoglu, M Altinbas, I Karadag, F B Basal, I Bilgetekin, O Sutcuoglu, O Yazici, N Ozdemir, A Ozet, Y Yildiz, S A Esen, G Ucar, D Uncu, B Dinc, M B Aykan, I Erturk, N Karadurmus, B Civelek and I Celik contributed to enrolling patients and interpreting data. M Dogan contributed to enrolling patients and revising the manuscript. Y Ergun contributed to study concept, study design and manuscript writing. O B Oksuzoglu contributed to study concept and revising the manuscript.

Acknowledgments

The authors thank all patients who participated in the study. Special thanks to Aydanur Sargın, Pinar Karacin, Arif Hakan Önder, Hatice Yiğit, Zeynep Sipahi Karslı, Ayşe Ocak Duran.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The study protocol was approved by the ethics committee of the HSU Dr Abdurrahman Yurtaslan Oncology Training & Research Hospital, and the study was undertaken in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent. Special permission for this study was obtained from the Ministry of Health.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. WHO coronavirus (COVID-19) dashboard (2021). https://covid19.who.int/
2. Yekedüz E, Utkan G, Ürün Y. A systematic review and meta-analysis: the effect of active cancer treatment on severity of COVID-19. *Eur. J. Cancer* 141, 92–104 (2020).
3. Karacin C, Acar R, Bal O *et al.* "Swords and Shields" against COVID-19 for patients with cancer at "clean" and "pandemic" hospitals: are we ready for the second wave? *Support. Care Cancer* 29(8), 4587–4593 (2021).
4. Akagunduz B, Ozer M, Karacin C, Atcı MM, Yıldırım HC, Unver E. Impact of coronaphobia on treatment and follow-up compliance of cancer patients. *Future Oncol.* 17(20), 2621–2629 (2021).
5. Garassino MC, Vyas M, de Vries EGE, Kanesvaran R, Giuliani R, Peters S. The ESMO call to action on COVID-19 vaccinations and patients with cancer: vaccinate. Monitor. Educate. *Ann. Oncol.* 32(5), 579–581 (2021).

• **Discusses the importance of COVID-19 vaccines in patients with cancer.**

6. Zhang Y, Zeng G, Pan H *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect. Dis.* 21(2), 181–192 (2021).

• **Discusses phase I and II CoronaVac trial results.**

7. Wu Z, Hu Y, Xu M *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect. Dis.* 21(6), 803–812 (2021).

• **Discusses phase I and II CoronaVac trial results.**

8. Mallapaty S. WHO approval of Chinese CoronaVac COVID vaccine will be crucial to curbing pandemic. *Nature* 594(7862), 161–162 (2021).

•• **Discusses news about approval of CoronaVac by WHO.**

9. Bosaeed M, Kumar D. Seasonal influenza vaccine in immunocompromised persons. *Hum. Vaccin. Immunother.* 14(6), 1311–1322 (2018).
10. Rousseau B, Loulergue P, Mir O *et al.* Immunogenicity and safety of the influenza A H1N1v 2009 vaccine in cancer patients treated with cytotoxic chemotherapy and/or targeted therapy: the VACANCE study. *Ann. Oncol.* 23(2), 450–457 (2012).
11. Sümbül AT, Yalçın Ş, Özet A *et al.* Turkish Medical Oncology Society COVID-19 pandemic advisory board updated recommendations for medical oncologists: included vaccination. *J. Oncol. Sci.* 7(1), 1–6 (2021).
12. European Society for Medical Oncology. ESMO statements for vaccination against COVID-19 in patients with cancer (2021). www.esmo.org/covid-19-and-cancer/covid-19-vaccination
13. Siemens Healthcare Diagnostics Atellica IM SARS-CoV-2 total (COV2T) ELISA assay for the detection of total antibodies to SARS-CoV-2. https://www.fda.gov/media/138442/download
14. Seyahi E, Bakhdiyarli G, Oztas M *et al.* Antibody response to inactivated COVID-19 vaccine (CoronaVac) in immune-mediated diseases: a controlled study among hospital workers and elderly. *Rheumatol. Int.* 41(8), 1429–1440 (2021).

•• **Discusses the immunogenicity of CoronaVac in patients with rheumatological disease receiving immunomodulator drugs.**

15. Agha M, Blake M, Chilleo C, Wells A, Haidar G. Suboptimal response to COVID-19 mRNA vaccines in hematologic malignancies patients. *medRxiv* doi:10.1101/2021.04.06.21254949 (2021) (Epub ahead of print).

•• **Discusses the immunogenicity of mRNA vaccines in cancer patients.**

16. Herishanu Y, Avivi I, Aharon A *et al.* Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood* 137(23), 3165–3173 (2021).

•• **Discusses the immunogenicity of mRNA vaccines in cancer patients.**

17. Massarweh A, Eliakim-Raz N, Stemmer A *et al.* Evaluation of seropositivity following BNT162b2 messenger RNA vaccination for SARS-CoV-2 in patients undergoing treatment for cancer. *JAMA Oncol.* doi:10.1001/jamaoncol.2021.2155 (2021) (Epub ahead of print).

•• **Discusses the immunogenicity of mRNA vaccines in cancer patients.**

18. Bitterman R, Eliakim-Raz N, Vinograd I, Trestioreanu AZ, Leibovici L, Paul M. Influenza vaccines in immunosuppressed adults with cancer. *Cochrane Database Syst. Rev.* 2(2), CD008983 (2018).

19. Ikeda H, Togashi Y. Aging, cancer, and antitumor immunity. *Int. J. Clin. Oncol.* doi:10.1007/s10147-021-01913-z (2021) (Epub ahead of print).

20. Sprengholz P, Korn L, Eitze S, Betsch C. Allocation of COVID-19 vaccination: when public prioritisation preferences differ from official regulations. *J. Med. Ethics.* doi:10.1136/medethics-2021-107339 (2021) (Epub ahead of print).

21. Abid MB. Overlap of immunotherapy-related pneumonitis and COVID-19 pneumonia: diagnostic and vaccine considerations. *J. Immunother. Cancer* 9(4), e002307 (2021).

22. Waissengrin B, Agbarya A, Safadi E, Padova H, Wolf I. Short-term safety of the BNT162b2 mRNA COVID-19 vaccine in patients with cancer treated with immune checkpoint inhibitors. *Lancet Oncol.* 22(5), 581–583 (2021).

4.12. CoronaVac aumenta em 70% anticorpos contra Covid-19 em pacientes imunossuprimidos, afirma estudo do HC

Pacientes com doenças reumatológicas autoimunes apresentaram um aumento de 70,4% no nível de anticorpos contra o vírus SARS-CoV-2 duas semanas após receberem a segunda dose da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19. Além de aumentar a soroconversão dos pacientes imunossuprimidos, a CoronaVac também elevou em 56,3% a quantidade de anticorpos neutralizantes.

As conclusões são de um estudo realizado pelo Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) com 910 pessoas e estão descritas no artigo Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial, divulgado na publicação científica Nature Medicine.

O resultado é extremamente positivo porque mostra que a CoronaVac não só é bem aceita pelo organismo de pacientes imunossuprimidos (que têm mais dificuldade para produzir anticorpos), como gera um alto nível de anticorpos de defesa e neutralizantes. A pesquisa do HC mostra não só que a CoronaVac é segura nesse público, como também eficaz.

"Trata-se do maior estudo já realizado no mundo com pacientes imunossuprimidos de doenças reumatológicas", afirma a diretora clínica do HCFMUSP, Eloisa Bonfá. "O acréscimo no nível de anticorpos é muito relevante e mostra que a CoronaVac conferiu uma proteção importante entre os imunossuprimidos", completa.

Outro dado que atesta a segurança da CoronaVac é a ausência de reações adversas nos vacinados. "Não tivemos nenhum caso de efeito colateral grave ou moderado entre os pacientes, mesmo sabendo que isso poderia ser esperado entre imunossuprimidos. Só tivemos efeitos colaterais leves. A CoronaVac é uma vacina altamente segura", assinala Eloisa.

De acordo com a diretora do hospital, os 910 pacientes imunossuprimidos participantes da pesquisa foram vacinados em dois dias. Pouco depois da segunda dose, quando os anticorpos ainda estavam em produção, houve 33 casos de Covid-19; 40 dias depois, esse número havia caído para seis casos.

Por que esse resultado é tão relevante?

O resultado de soroconversão (capacidade de produzir anticorpos) da CoronaVac nos pacientes imunossuprimidos do HCFMUSP é surpreendente, especialmente na comparação com o grupo controle, formado por pessoas sem deficiências de imunidade. O nível de anticorpos de defesa gerados nos imunossuprimidos foi de 70,4%, enquanto no grupo controle foi de 95%; já o nível de anticorpos neutralizantes foi de 56,3% nos imunossuprimidos, e de 79,3% no grupo controle.

Pessoas com doenças reumáticas autoimunes são geralmente tratadas com corticoides combinados com imunossupressores. Ou seja, seus tratamentos costumam envolver medicações que atuam justamente para reprimir o sistema imunológico, impedindo que ele atue de forma a agravar a doença autoimune.

A consequência disso é que imunossuprimidos têm uma menor capacidade de produzir anticorpos. Por isso, seus organismos são mais suscetíveis a contrair doenças infecciosas, como é o caso da Covid-19, e evoluir para casos graves. Antes da pesquisa do HCFMUSP, esse público estava impedido de tomar a vacina e só podia contar com medidas ainda em desenvolvimento, como o soro anti-Covid.

As doenças reumatológicas autoimunes compreendem diversas síndromes, como miosite autoimune, fasciíte eosinofílica, doença mista do tecido conjuntivo, policondrite recidivante, síndrome de Sjögren, lúpus eritematoso sistêmico e esclerodermia.

Sobre a Nature Medicine

Uma das publicações científicas mais conceituadas do mundo, conhecida entre pesquisadores pelo seu rigor, a Nature Medicine divulga estudos focados no desenvolvimento de novas tecnologias e conhecimentos ligados à medicina contemporânea.

Publicado em: 30/07/2021

ARTICLES
https://doi.org/10.1038/s41591-021-01469-5
nature medicine

Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial

Ana C. Medeiros-Ribeiro[1,6], Nadia E. Aikawa[1,2,6], Carla G. S. Saad[1], Emily F. N. Yuki[1], Tatiana Pedrosa[1], Solange R. G. Fusco[1], Priscila T. Rojo[1], Rosa M. R. Pereira[1], Samuel K. Shinjo[1], Danieli C. O. Andrade[1], Percival D. Sampaio-Barros[1], Carolina T. Ribeiro[1], Giordano B. H. Deveza[1], Victor A. O. Martins[1], Clovis A. Silva[2], Marta H. Lopes[3], Alberto J. S. Duarte[4], Leila Antonangelo[4], Ester C. Sabino[3,5], Esper G. Kallas[3], Sandra G. Pasoto[1] and Eloisa Bonfa[1]✉

CoronaVac, an inactivated SARS-CoV-2 vaccine, has been approved for emergency use in several countries. However, its immunogenicity in immunocompromised individuals has not been well established. We initiated a prospective phase 4 controlled trial (no. NCT04754698, CoronavRheum) in 910 adults with autoimmune rheumatic diseases (ARD) and 182 age- and sex-frequency-matched healthy adults (control group, CG), who received two doses of CoronaVac. The primary outcomes were reduction of ≥15% in both anti-SARS-CoV-2 IgG seroconversion (SC) and neutralizing antibody (NAb) positivity 6 weeks (day 69 (D69)) after the second dose in the ARD group compared with that in the CG. Secondary outcomes were IgG SC and NAb positivity at D28, IgG titers and neutralizing activity at D28 and D69 and vaccine safety. Prespecified endpoints were met, with lower anti-SARS-Cov-2 IgG SC (70.4 versus 95.5%, $P < 0.001$) and NAb positivity (56.3 versus 79.3%, $P < 0.001$) at D69 in the ARD group than in the CG. Moreover, IgG titers (12.1 versus 29.7, $P < 0.001$) and median neutralization activity (58.7 versus 64.5%, $P = 0.013$) were also lower at D69 in patients with ARD. At D28, patients with ARD presented with lower IgG frequency (18.7 versus 34.6%, $P < 0.001$) and NAb positivity (20.6 versus 36.3%, $P < 0.001$) than that of the CG. There were no moderate/severe adverse events. These data support the use of CoronaVac in patients with ARD, suggesting reduced but acceptable short-term immunogenicity. The trial is still ongoing to evaluate the long-term effectiveness/immunogenicity.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected millions of people around the world[1]. Brazil is among those countries with the highest numbers of confirmed cases of, and deaths from, SARS-CoV-2 (refs. [1,2]), with >430,000 deaths registered and approximately 15 million cases as of May 2021 (ref. [1]). A second infection wave was driven by the Gamma coronavirus variant[3], which is considered to be 2.5-fold more contagious than the original strain[4] and possibly associated with a higher risk for hospitalization and intensive care unit admission in patients younger than 60 years of age[5]. This second peak in March and April 2021 resulted in more than double the reported coronavirus disease 2019 (COVID-19) cases of the first peak in 2020 (ref. [6]). Vaccines are therefore essential in regard to reducing COVID-19 mortality and morbidity.

Although phase 3 clinical trials results are still being consolidated in China, Hong Kong, Indonesia, Brazil, Chile, Philippines and Turkey[7], CoronaVac, an inactivated virus vaccine against SARS-CoV-2, has received emergency use approval by the World Health Organization (WHO) in several countries, including three of the six most populated in the world—Brazil, China and Turkey—which are important for the global control of this disease. At the time of this submission, CoronaVac has accounted for approximately 75% of the vaccines administered in Brazil. It can be kept refrigerated[8], a great advantage for deployment in developing countries. In addition, the more traditional technology using the whole virus may have the benefit of a broader immune response compared to the other vaccine platforms using only the Spike protein. This may be relevant for control of SARS-CoV-2 variants containing mutations in the Spike protein, which have been documented in Brazil[3,9]. Cross-reactive humoral immune responses against the Gamma and Zeta variants were achieved in healthy volunteers vaccinated with CoronaVac in a phase 3 clinical trial conducted in Brazil[10,11].

However, the reported 50.7% efficacy in prevention of mild COVID-19 in the phase 3 clinical trial[10] raises concerns about the immunogenicity of CoronaVac in immunosuppressed patients, who number millions, including those with autoimmune diseases, neoplasia, transplant recipients and those living with human immunodeficiency virus (HIV) among other groups, with an estimated prevalence in the United States of 2.7% of the population[12]. A recent letter reported a greatly reduced anti-Spike antibody response after two doses of SARS-CoV-2 mRNA 1273 or BNT162b2 vaccination in solid organ transplant recipients[13,14]. Previous studies on COVID-19 vaccine immunogenicity in patients with ARD have suggested slightly reduced humoral responses, but have been

[1]Rheumatology Division, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil. [2]Pediatric Rheumatology Unit, Instituto da Criança, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil. [3]Infectious Disease Department, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil. [4]Central Laboratory Division, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil. [5]Instituto de Medicina Tropical, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil. [6]These authors contributed equally: Ana C. Medeiros-Ribeiro, Nadia E. Aikawa. ✉e-mail: eloisa.bonfa@hc.fm.usp.br

limited by the absence of a control group, small numbers of patients with ARD, and the fact that neutralizing antibodies have not necessarily been assessed[15–19]. In addition, most earlier studies evaluated immunogenicity following messenger RNA vaccines and thus CoronaVac immunogenicity in immunocompromised individuals remains unclear[13–19]. Importantly, immunocompromised patients are at high risk for infectious diseases due to immune dysregulation and treatment regimens. In addition, they may fulfill criteria for prioritization in the context of limited vaccine supply, since COVID-19 severity is associated not only with highly prevalent comorbidities in these patients but also with disease activity[10–24]. Moreover, an immunocompromised state was reported to be associated with prolonged SARS-CoV-2 shedding[25], reduced SARS-CoV-2 virus clearance and enhanced viral genomic evolution[26], emphasizing the relevance of the vaccine for this group of patients in reducing transmission and preventing the emergence of new variants.

In this context, the present study aimed to prospectively evaluate the immunogenicity (anti-SARS-CoV-2 IgG and neutralizing antibodies) and safety of CoronaVac in a large cohort of patients with ARD compared with an age- and sex-frequency-matched control group without these conditions and with no immunosuppressive therapy. As an exploratory outcome, we further checked for incident symptomatic cases, as confirmed by real-time reverse transcriptase–PCR (RT–PCR) for SARS-CoV-2 and the presence of variants of concern (VOC) (Gamma, Alpha and Beta lineages).

Results

Study design and participants. This phase 4 prospective controlled clinical trial (CoronavRheum clinicaltrials.gov no. NCT04754698) was conducted at a single tertiary center in Brazil.

The primary outcome was humoral immunogenicity, assessed by two coprimary endpoints: a minimum of 15% reduction in SC rates of anti-S1/S2 SARS-CoV-2 IgG and the presence of NAb 6 weeks after administration of the second vaccine dose (D69) in patients with ARD compared to controls, based on a previous study of primary vaccination with the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of patients with ARD[27].

Secondary immunogenicity outcomes were: anti-S1/S2 IgG seroconversion and presence of NAb at D28 (after vaccine first dose); geometric mean titers of anti-S1/S2 IgG and their factor increase in geometric mean titer (FI-GMT) at D28 and D69; and median (interquartile range, IQR) neutralizing activity of NAb at D28 and D69. Another secondary outcome was safety related to the vaccine doses. Exploratory outcomes were factors associated with anti-SARS-CoV-2 IgG SC and NAb positivity at D69, and incident COVID-19 case evaluation for a total of 80 days (from day of vaccination (D0) to 10 days after the second dose (D39) and thereafter for the following 40 days (from D40 to D79)).

A total of 1,418 patients with ARD were invited to join the study, but 225 were excluded according to established criteria: acute febrile illness/symptoms of suspected COVID-19 on the day of vaccination or with real-time RT–PCR-confirmed COVID-19 <4 weeks before D0 ($n=24$); demyelinating disease ($n=1$); previous vaccination with any COVID-19 vaccine ($n=25$); inactivated virus vaccine up to 2 weeks before D0 ($n=1$); individuals who did not consent to participate in the study ($n=161$); and hospitalization for general reasons ($n=13$). Subsequently, 542 healthy adult controls were invited but 50 individuals refused to participate. The remaining 1,193 patients with ARD and 492 controls received the first dose of CoronaVac, but 232 (19.4%) patients with ARD and 191 (38.8%) controls had positive baseline IgG serology and/or NAb and were thus excluded from this analysis. The remaining 961 patients with ARD and 301 controls with negative serology were then frequency matched in a 5/1 ratio (five ARD/one control) by age (maximal variation ± 5 years) and sex, with 910 patients with ARD and 182 healthy adults (CG) comprising the final study groups

Table 1 | Baseline characteristics of patients with ARD and CG

	ARD ($n=910$)	CG ($n=182$)	*P* value
Demographics			
Current age (years)	51 (40–60)	50 (41–60)	0.985
Female sex	700 (76.9)	140 (76.9)	>0.999
Caucasian race	482 (53.0)	82 (45.1)	0.051
Comorbidities			
Systemic arterial hypertension	400 (44.0)	55 (30.2)	0.001
Diabetes mellitus	106 (11.6)	28 (15.4)	0.161
Dyslipidemia	246 (27.0)	14 (7.7)	<0.001
Obesity	295 (32.4)	58 (31.9)	0.954
Chronic cardiomyopathy	52 (5.7)	3 (1.6)	0.024
Chronic renal disease	44 (4.8)	0	0.001
Current smoking	84 (9.2)	21 (11.0)	0.461
Chronic obstructive pulmonary disease	13 (1.4)	2 (1.1)	>0.999
Asthma	36 (4.0)	6 (3.3)	0.673
Interstitial lung disease	78 (8.6)	0	<0.001
Pulmonary hypertension	13 (1.4)	0	0.142
Hematologic disease	3 (0.3)	0	>0.999
Hepatic disease	39 (4.3)	0	0.001
Current cancer	8 (0.9)	0	0.365
Stroke	34 (3.7)	0	0.004
Current tuberculosis	2 (0.2)	0	>0.999
HIV	0	0	-
ARD			
Chronic inflammatory arthritis (RA, axSpA, PsA)	451 (49.6)	-	-
Other ARD (SLE, primary vasculitis, SSc, pSSj, IIM, PAPS)	459 (50.4)	-	-
Current therapy			
Prednisone	348 (38.2)	-	-
Prednisone dose, mg	5 (5–10)	-	-
Prednisone ≥20 mg day^{-1}	32 (3.5)	-	-
Hydroxychloroquine	269 (29.6)	-	-
Sulfasalazine	73 (8.0)	-	-
Immunosuppressive drugs	573 (63.0)	-	-
Methotrexate	229 (25.2)	-	-
Leflunomide	130 (14.3)	-	-
Mycophenolate mofetil	119 (13.1)	-	-
Azathioprine	109 (12.0)	-	-
Tofacitinib	19 (2.1)	-	-
Cyclophosphamide	10 (1.1)	-	-
Tacrolimus	10 (1.1)	-	-
Cyclosporine	9 (1.0)	-	-
Biologic therapy	321 (35.3)	-	-
TNFi	138 (15.2)	-	-
Abatacept	51 (5.6)	-	-
Tocilizumab	50 (5.5)	-	-
Belimumab	30 (3.3)	-	-
Secukinumab	29 (3.2)	-	-
Rituximab	19 (2.1)	-	-
Ustekinumab	5 (0.5)	-	-

Results are expressed as median (IQR) and *n* (%). Continuous data were compared using the Mann–Whitney *U*-test, and categorical variables with the chi-square or Fisher's exact test, as appropriate, always as two-sided analyses.

Table 2 | Seroconversion rates at D28 and D69; anti-SARS-CoV-2 S1/S2 IgG titers before (D0) and after the first (D28) and second dose (D69) of CoronaVac vaccination in patients with ARD and CG

	SC		GMT (AU ml^{-1})			FI-GMT	
	D28	D69	D0	D28	D69	D0 to D28	D0 to D69
ARD, $n = 859$	161 (18.7)	605 (70.4)	2.2 (2.2–2.3)	5.1 (4.7–5.5)	27.0 (24.7–29.5)	2.3 (2.1–2.5)	12.1 (11.0–13.2)
CG, $n = 179$	62 (34.6)	171 (95.5)	2.3 (2.1–2.4)	10.3 (8.5–12.5)	67.0 (59.8–74.9)	4.6 (3.9–5.4)	29.7 (26.3–33.5)
P (ARD versus CG)	<0.0001	<0.0001	>0.9990	<0.0010	<0.0010	<0.0010	<0.0010

SC is defined as post-vaccination titer ≥15 AU ml^{-1} by indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG. Frequencies of SC are presented as number (%), and were compared using a two-sided chi-square test between ARD and CG at prespecified time points (D28 and D69). IgG antibody titers and FI-GMT are expressed as geometric means with 95% CI. Data regarding IgG titers were analyzed using ANOVA with repeated measures and two factors (two groups (ARD versus CG) at three time points (D0, D28 and D69)), followed by Bonferroni's multiple comparisons at ln-transformed data (Supplementary Table 1). The behavior of IgG titers was different for ARD and CG groups between D28 and D69: mean titers increased at each time point for ARD and CG ($P < 0.001$). FI-GMT values were compared using the Mann–Whitney *U*-test for intergroup comparisons in ln-transformed data at prespecified time points (D28 and D69). All analyses were two-sided.

Table 3 | Frequency of NAb and median percentage of neutralizing activity in positive cases, after the first (D28) and second dose (D69) of CoronaVac vaccination in patients with ARD in comparison to CG

	D28		D69	
	Subjects with positive NAb, *n* (%)	Neutralizing activity (%) median (IQR)	Subjects with positive NAb, *n* (%)	Neutralizing activity (%) median (IQR)
ARD, $n = 859$	177 (20.6)	42.6 (35.8–60.4)	484 (56.3)	58.7 (43.1–77.2)
CG, $n = 179$	65 (36.3)	45 (34 .5–71.1)	142 (79.3)	64.5 (48.4–81.4)
P (ARD versus CG)	<0.0001	0.4900	<0.0001	0.0130

Frequencies of subjects with positive NAb are expressed as number (%). Positivity for NAb was defined as neutralizing activity ≥30% (cPass sVNT Kit). Data were compared using a two-sided chi-square test between ARD and CG at prespecified time points (D28 and D69). Percentage of neutralizing activity among subjects with positive NAb is expressed as median (IQR). Data were compared using a two-sided Mann–Whitney *U*-test for comparison between ARD and CG, at prespecified time points (D28 and D69).

(Extended Data Fig. 1). Enrollment and vaccination occurred on the same day for each participant. The first subject was enrolled and vaccinated on 9 February 2021 and the last participant was enrolled and vaccinated on 24 February 2021. The majority ($n = 1{,}017$, 93.1%) of patients and controls were recruited and vaccinated on 9 or 10 February 2021, with no differences between the ARD and CG groups (92.7 versus 95.1%, $P = 0.261$). Patients and controls were followed until D79 after the first vaccine dose (D0) for analysis of immunogenicity and incident cases in this study. The trial is no longer recruiting, but it is still ongoing for long-term effectiveness and immunogenicity.

Patients with ARD had the following disease diagnoses: chronic inflammatory arthritis (CIA) ($n = 451$, 49.6%), rheumatoid arthritis (RA) ($n = 256$, 28.1%), axial spondyloarthritis (axSpA) ($n = 106$, 11.6%) or psoriatic arthritis (PsA) ($n = 89$, 9.8%) and other systemic ARD ($n = 459$, 50.4%), systemic lupus erythematosus (SLE) ($n = 232$, 25.5%), primary vasculitis ($n = 66$, 7.3%), primary Sjögren's syndrome (pSSj) ($n = 42$, 4.6%), systemic sclerosis (SSc) ($n = 41$, 4.5%), idiopathic inflammatory myopathies (IIM) ($n = 41$, 4.5%) and primary antiphospholipid syndrome (PAPS) ($n = 37$, 4.1%) (Table 1). The control group ($n = 182$, CG) included hospital cleaning and general maintenance services workers ($n = 109$, 59.9%), health professionals ($n = 45$, 24.7%) and hospital administrative services employees or their relatives ($n = 28$, 15.4%).

The ARD and CG groups had comparable median ages (51 versus 50 years, $P = 0.985$) and enrollment of females (76.9 versus 76.9%, $P > 0.999$) (Table 1). Frequencies of comorbidity were higher in ARD, particularly systemic arterial hypertension (44.0 versus 30.2%, $P = 0.001$), dyslipidemia (27.0 versus 7.7%, $P < 0.001$), interstitial lung disease (8.6 versus 0%, $P < 0.001$), cardiomyopathy (5.7 versus 1.6%, $P = 0.024$) and chronic renal disease (4.8 versus 0%, $P = 0.001$) (Table 1). A total of 348 (38.2%) patients with ARD were receiving ongoing treatment with prednisone and 573 (63.0%) were using immunosuppressive drugs. Of those patients treated with immunosuppressive drugs, 25.2% were using methotrexate, 14.3% leflunomide, 13.1% mycophenolate mofetil, 12% azathioprine and <3% others. Of those 321 (35.3%) patients were being treated using biologic therapies, 15.2% were using tumor necrosis factor inhibitor (TNFi), 5.6% abatacept, 5.5% tocilizumab, 3.3% belimumab, 3.2% secukinumab and <3% others (Table 1).

For the primary outcome analysis of immunogenicity, we excluded 38 (4.2%) participants (35 patients with ARD and three CG participants) with real-time RT–PCR-confirmed COVID-19 after either the first or second dose of vaccine until D69, and 16/910 (1.5%) patients who did not attend the final visit (D69), including two deaths not related to COVID-19.

Primary immunogenicity outcomes. Humoral response parameters in the remaining 859 patients with ARD and 179 controls, all with negative anti-SARS-CoV-2 S1/S2 IgG antibodies and NAb pre-vaccination, are shown in Tables 2 and 3.

The study met the primary outcomes, defined as a minimum of 15% reduction in anti-S1/S2 SARS-CoV-2 IgG SC and in the presence of NAb in patients with ARD compared to CG at 6 weeks (D69) after the second dose. Analysis of the SARS-CoV-2 S1/S2 IgG response at D69 revealed a lower SC rate in patients with ARD (70.4 versus 95.5%, $P < 0.001$). Similarly, NAb positivity was lower in patients with ARD compared to controls (56.3 versus 79.3%, $P < 0.001$).

Secondary outcomes. Secondary immunogenicity outcomes defined by anti-SARS-CoV-2 IgG SC at D28, as well as IgG GMT and FI-GMT at D28 and D69, are presented in Table 2 and Fig. 1. SARS-CoV-2 cPass virus NAb positivity at D28 and median activity of NAb at D28 and D69 were also secondary outcomes (Table 3).

A minority of participants in both groups developed anti-SARS-CoV-2 IgG antibodies after the first dose (D28), with a lower frequency and level in patients with ARD compared to CG (161 (18.7%) versus 62 (34.6%), $P < 0.001$) and FI-GMT (2.3 (95% confidence interval (CI) 2.1–2.5) versus 4.6 (95% CI 3.9–5.4), $P < 0.001$). The SC rates doubled after the second vaccine dose, with an

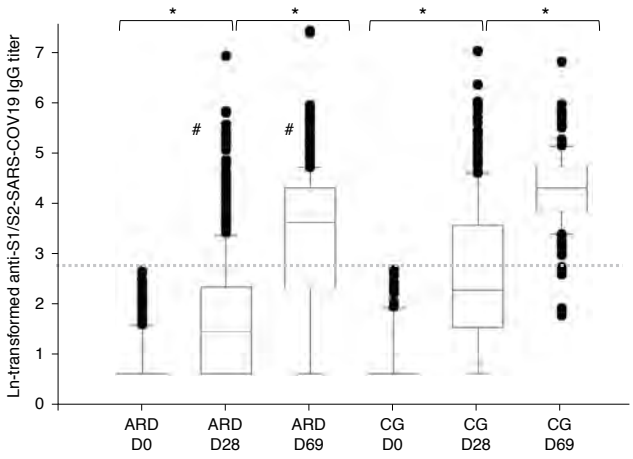


Fig. 1 | Anti-SARS-CoV-2 S1/S2 IgG titers of patients with ARD and subjects in CG at D0, D28 and D69. Box plots show the distribution of ln-transformed IgG titers over time. Data for each group (ARD, $n=859$ and CG, $n=179$) are presented at each time point as box plots: central values within boxes correspond to median (50th percentile, or Q2); the range between the lower (25th percentile, or Q1) and upper (75th percentile, or Q3) bounds of the boxes is the IQR. Whiskers represent scores outside IQR and ends in maximum (higher "calculated value" = Q3 + 1.5 x IQR) and minimum (lower "calculated value" = Q1 - 1.5 x IQR). Spots are outliers above the maximum or under the minimum values. The minimum possible value is 0.64 (ln 1.9, the value attributed to IgG titers ≤3.8 AU ml^{-1}). Data regarding IgG titers were analyzed using ANOVA with repeated measures and two factors (two groups (ARD versus CG), at three time points (D0, D28 and D69)), followed by Bonferroni's multiple comparison of ln-transformed data (Supplementary Table 1). Tests were always two-sided. The mean behavior of the ln-transformed IgG titers was different in ARD and CG groups at D28 ($P<0.001$) and D69 ($P<0.001$). Mean titers increased at each time point for ARD and CG ($^{*}P<0.001$). At D28 and D69 evaluations, patients with ARD presented lower mean titers than CG ($^{\#}P<0.001$). ARD and CG were comparable only at D0 ($P>0.999$). Dotted line denotes the cut-off level for positivity (ln 15 AU ml^{-1} = 2.71 by Indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG).

increase of more than fivefold in GMT (FI-GMT) for both groups (Table 2 and Fig. 1).

According to Bonferroni's multiple comparison, the mean behavior of the neperian logarithm (ln)-transformed IgG titers was different in the ARD and CG groups between D28 and D69 ($P<0.001$). Mean IgG titers were similar at D0 in both groups ($P>0.999$) and increased at each time point for ARD and CG ($P<0.001$). At the D28 and D69 evaluations, patients with ARD presented lower mean titers than CG ($P<0.001$) (Table 2, Fig. 1 and Supplementary Table 1).

Analysis of the dynamics of NAb detection showed that after the first dose (D28), a minority of participants had positive antibodies and patients with ARD had lower frequencies (177 (20.6%) versus 65 (36.3%), $P<0.001$), but with similar median (IQR) activity (42.6% (35.8–60.4) versus 45% (34.5–71.1), $P=0.490$) compared with CG (Table 3). At D69, lower median (IQR) neutralization activity (58.7% (43.1–77.2) versus 64.5% (48.4–81.4), $P=0.013$) was observed.

Vaccine tolerance and safety. Vaccine safety analysis, another secondary outcome, is illustrated in Table 4. No moderate/severe adverse events (AEs) related to the vaccine were reported. After the first dose, the most frequently reported vaccine reactions in patients with ARD and CG were pain at the injection site (19.8 versus 17.0%, $P=0.388$), headache (20.2 versus 11.0%, $P=0.003$) and somnolence (13.6 versus 10.4%, $P=0.243$). Overall reactions were more frequently reported in patients with ARD than CG (50.5 versus 40.1%, $P=0.011$), including arthralgia (13.5 versus 6.0%, $P=0.005$), back pain (9.8 versus 4.9%, $P=0.037$), malaise (9.5 versus 4.4%, $P=0.026$), nausea (6.1 versus 2.2%, $P=0.032$) and sweating (5.6 versus 1.1%, $P=0.007$). After the second dose, patients with ARD reported less local itching (2.7 versus 5.5%, $P=0.047$) and more sweating (5.3 versus 1.1%, $P=0.010$) (Table 4).

Factors associated with lower anti-SARS-CoV-2 IgG SC and NAb positivity in patients with ARD. We also analyzed factors associated with anti-SARS-CoV-2 IgG SC and NAb positivity as exploratory outcomes (Table 5). Patients with negative anti-SARS-CoV-2 IgG after two doses of CoronaVac (D69) were of older age ($P<0.001$), with a higher frequency of females (81.9 versus 74.7%, $P=0.023$) compared to those with positive anti-SARS-CoV-2 IgG. Non-seroconverters used the following therapies more often: prednisone (55.9 versus 31.1%, $P<0.001$) and prednisone ≥20 mg day^{-1} (5.5 versus 2.6%, $P=0.037$); immunosuppressants (81.9 versus 54.5%, $P<0.001$), particularly methotrexate (34.6 versus 21.7%, $P<0.001$) and mycophenolate mofetil (24.4 versus 7.9%, $P<0.001$); and biologic therapy (44.1 versus 32.2%, $P=0.001$), especially abatacept (11.4 versus 3.3%, $P<0.001$) and rituximab (4.3 versus 1.3%, $P=0.006$) (Table 5). Multivariate logistic regression analysis (Supplementary Table 2) was performed using as dependent variables SC or the presence of NAb at D69 (primary endpoint), and as independent variables those with $P<0.2$ in the univariate analysis presented in Table 5. This analysis revealed that age ≥60 years (odds ratio (OR) = 0.51; 95% CI 0.36–0.74, $P<0.001$), prednisone (OR = 0.40; 95% CI 0.28–0.56, $P<0.001$), methotrexate (OR = 0.42; 95% CI 0.29–0.61, $P<0.001$), mycophenolate mofetil (OR = 0.15; 95% CI 0.09–0.24, $P<0.001$), TNFi (OR = 0.41; 95% CI 0.26–0.64, $P<0.001$), abatacept (OR = 0.24; 95% CI 0.13–0.46, $P<0.001$) and rituximab (OR = 0.34; 95% CI 0.13–0.93, $P=0.036$) were associated with the absence of SC in patients with ARD (Supplementary Table 2).

Similarly, patients with negative NAb after complete vaccination (D69) were older (52 (43–62) versus 49 (39–59) years, $P<0.001$) than those with positive NAb. Patients with negative NAb at D69 were more frequently ≥60 years of age (32.5 versus 22.5%, $P=0.001$) and using prednisone (49.3 versus 30%, $P<0.001$), immunosuppressants (72.5 versus 55%, $P<0.001$), including methotrexate (30.4 versus 21.7%, $P=0.004$) and mycophenolate mofetil (17.9 versus 8.9%, $P<0.001$) or biologic therapy (41.3 versus 31.4%, $P=0.003$), including abatacept (8.0 versus 3.9%, $P=0.011$) and rituximab (4.0 versus 0.8%, $P=0.002$) (Table 5). Multivariate analysis identified age ≥60 years (OR = 0.65; 95% CI 0.46–0.91, $P=0.011$), prednisone (OR = 0.48; 95% CI 0.35–0.65, $P<0.001$), methotrexate (OR = 0.67, 95% CI 0.47–0.95, $P=0.024$), mycophenolate mofetil (OR = 0.33; 95% CI 0.21–0.53, $P<0.001$) and rituximab (OR = 0.28; 95% CI 0.09–0.87, $P=0.028$) as associated with the absence of neutralizing activity in patients with ARD (Supplementary Table 2).

COVID-19 incident cases. For the analysis of incident cases, another exploratory outcome was used—participants were followed during strictly equivalent time periods of 40 days before and after full vaccination: from D0 to D39 and from D40 to D79. Therefore, the evaluation period for incident cases was extended to 10 days (D79) after the final immunogenicity analysis (D69). A total of 39 incident symptomatic, RT–PCR-confirmed COVID-19 cases among patients with ARD and CG were observed during the evaluation periods, with no significant difference between groups (4.0 versus 1.6%, $P=0.186$). The frequency of cases occurring

Table 4 | Adverse events following CoronaVac vaccination in patients with ARD and CG

	After vaccine first dose			After vaccine second dose		
	ARD ($n = 909$)	CG ($n = 182$)	*P* value	ARD ($n = 893$)	CG ($n = 181$)	*P* value
No symptoms	450 (49.5)	109 (59.9)	0.011	545 (61.0)	118 (65.2)	0.293
Local reactions (at the injection site)	213 (23.4)	36 (19.8)	0.284	154 (17.2)	32 (17.7)	0.888
Pain	180 (19.8)	31 (17.0)	0.388	125 (14.0)	30 (16.6)	0.368
Erythema	25 (2.8)	5 (2.7)	0.998	23 (2.6)	3 (1.7)	0.602
Swelling	43 (4.7)	12 (6.6)	0.294	45 (5.0)	10 (5.5)	0.787
Bruising	28 (3.1)	6 (3.3)	0.878	23 (2.6)	2 (1.1)	0.232
Pruritus	28 (3.1)	4 (2.2)	0.637	24 (2.7)	10 (5.5)	0.047
Induration	56 (6.2)	4 (2.2)	0.032	41 (4.6)	12 (6.6)	0.248
Systemic reactions	392 (43.3)	61 (33.5)	0.014	298 (33.4)	56 (30.9)	0.526
Fever	25 (2.8)	5 (2.7)	0.998	23 (2.6)	7 (3.9)	0.336
Malaise	86 (9.5)	8 (4.4)	0.026	80 (9.0)	15 (8.3)	0.772
Somnolence	124 (13.6)	19 (10.4)	0.243	83 (9.3)	15 (8.3)	0.668
Lack of appetite	37 (4.1)	7 (3.8)	0.888	37 (4.1)	7 (3.9)	0.864
Nausea	55 (6.1)	4 (2.2)	0.032	58 (6.5)	13 (7.2)	0.734
Vomiting	14 (1.5)	1 (0.5)	0.488	11 (1.2)	2 (1.1)	>0.999
Diarrhea	56 (6.2)	9 (4.9)	0.527	56 (6.3)	12 (6.6)	0.857
Abdominal pain	44 (4.8)	7 (3.8)	0.562	43 (4.8)	10 (5.5)	0.688
Vertigo	64 (7.0)	9 (4.9)	0.302	46 (5.2)	9 (5.0)	0.921
Tremor	22 (2.4)	1 (0.5)	0.155	20 (2.2)	2 (1.1)	0.562
Headache	184 (20.2)	20 (11.0)	0.003	130 (14.6)	33 (18.2)	0.209
Fatigue	99 (10.9)	14 (7.7)	0.196	95 (10.6)	22 (12.2)	0.550
Sweating	51 (5.6)	2 (1.1)	0.007	47 (5.3)	2 (1.1)	0.010
Myalgia	81 (8.9)	10 (5.5)	0.128	78 (8.7)	17 (9.4)	0.776
Muscle weakness	68 (7.5)	7 (3.8)	0.077	68 (7.6)	11 (6.1)	0.470
Arthralgia	123 (13.5)	11 (6.0)	0.005	93 (10.4)	13 (7.2)	0.184
Back pain	89 (9.8)	9 (4.9)	0.037	77 (8.6)	19 (10.5)	0.420
Cough	63 (6.9)	8 (4.4)	0.206	57 (6.4)	12 (6.6)	0.902
Sneezing	75 (8.3)	9 (4.9)	0.127	87 (9.7)	18 (9.9)	0.933
Coryza	75 (8.3)	13 (7.1)	0.616	76 (8.5)	17 (9.4)	0.701
Stuffy nose	52 (5.7)	8 (4.4)	0.474	55 (6.2)	11 (6.1)	0.967
Sore throat	67 (7.4)	7 (3.8)	0.084	60 (6.7)	11 (6.1)	0.751
Shortness of breath	29 (3.2)	6 (3.3)	0.941	23 (2.6)	6 (3.3)	0.576
Conjunctivitis	12 (1.3)	0	0.235	9 (1.0)	2 (1.1)	>0.999
Pruritus	33 (3.6)	3 (1.6)	0.253	39 (4.4)	6 (3.3)	0.519
Skin rash	9 (1.0)	3 (1.6)	0.433	14 (1.6)	0	0.090

Results are presented as *n* (%) and compared with the chi-square or Fisher's exact test, as appropriate, always as two-sided analyses.

between D0 and D39 (until 10 days after the second dose) was higher compared to D40–D79 (33/1,092 (3.0%) versus 6/1,057 (0.6%), $P < 0.0001$). Four patients with ARD were hospitalized (<10 days after the second dose) and none died from COVID-19. There was no hospitalizations or deaths associated with COVID-19 in the CG. Eighteen symptomatic participants with RT–PCR-confirmed COVID-19 were genotyped in our service; 83.3% of infections were due to Gamma variants, 5.6% to Alpha and 11.1% to other variants. SARS-CoV-2 genotyping could not be performed in the remaining 21 symptomatic participants because they were unable to attend our center due to the long traveling distance involved, and therefore their samples were collected for RT–PCR at an independent laboratory near to their home.

Finally, we considered environmental factors that could influence SARS-CoV-2 infection risk in those participants who answered the targeted questions about their exposure. Patients with ARD reported higher adherence to social isolation 69.5 versus 21.7%, $P < 0.001$) with lower household contact with infected people (4.6 versus 15.5%, $P = 0.0001$) and lower use of public transportation (47.7 versus 81.7%, $P < 0.001$) compared to CG. The numbers of people living in the same home were comparable in both groups (median of two).

Discussion

Vaccination of immunosuppressed patients, who were excluded from phase 3 vaccine trials, is of the utmost importance since

Table 5 | Baseline characteristics of patients with ARD with and without SC for anti-SARS-CoV-2 S1/S2 IgG antibodies and with and without NAb after two doses of CoronaVac vaccination

	ARD patients without SC ($n = 254$)	ARD patients with SC ($n = 605$)	*P* value	ARD patients without NAb ($n = 375$)	ARD patients with NAb ($n = 484$)	*P* value
Demographics						
Current age (years)	53 (45–63)	49 (39–59)	<0.001	52 (43–62)	49 (39–59)	<0.001
Age ≥60 years	89 (35)	142 (23.5)	<0.001	122 (32.5)	109 (22.5)	0.001
Female sex	208 (81.9)	452 (74.7)	0.023	293 (78.1)	367 (75.8)	0.427
Caucasian race	144 (56.7)	312 (51.6)	0.170	213 (56.8)	243 (50.2)	0.055
ARD						
CIA	126 (49.6)	304 (50.2)	0.864	200 (53.3)	230 (47.5)	0.091
Other ARD	128 (50.4)	301 (49.8)		175 (46.7)	254 (52.5)	
Current therapy						
Prednisone	142 (55.9)	188 (31.1)	<0.001	185 (49.3)	145 (30.0)	<0.001
Prednisone dose (mg)	5 (5–10)	5 (5–10)	0.926	5 (5–10)	5 (5–10)	0.731
Prednisone ≥20 mg day^{-1}	14 (5.5)	16 (2.6)	0.037	15 (4)	15 (3.1)	0.476
Hydroxychloroquine	72 (28.3)	182 (30.1)	0.611	98 (26.1)	156 (32.2)	0.052
Sulfasalazine	10 (3.9)	61 (10.1)	0.003	24 (6.4)	47 (9.7)	0.081
Immunosuppressive drugs	208 (81.9)	330 (54.5)	<0.001	272 (72.5)	266 (55)	<0.001
Methotrexate	88 (34.6)	131 (21.7)	<0.001	114 (30.4)	105 (21.7)	0.004
Leflunomide	37 (14.6)	84 (13.9)	0.793	57 (15.2)	64 (13.2)	0.409
Mycophenolate mofetil	62 (24.4)	48 (7.9)	<0.001	67 (17.9)	43 (8.9)	<0.001
Azathioprine	31 (12.2)	69 (11.4)	0.739	40 (10.7)	60 (12.4)	0.433
Tofacitinib	3 (1.2)	15 (2.5)	0.301	10 (2.7)	8 (1.7)	0.304
Cyclophosphamide	2 (0.8)	7 (1.2)	>0.999	3 (0.8)	6 (1.2)	0.739
Tacrolimus	4 (1.6)	6 (1.0)	0.493	4 (1.1)	6 (1.2)	0.815
Cyclosporine	4 (1.6)	4 (0.7)	0.245	6 (1.6)	2 (0.4)	0.085
Biologic therapy	112 (44.1)	195 (32.2)	<0.001	155 (41.3)	152 (31.4)	0.003
TNFi	45 (17.7)	86 (14.2)	0.193	63 (16.8)	68 (14.0)	0.266
Abatacept	29 (11.4)	20 (3.3)	<0.001	30 (8.0)	19 (3.9)	0.011
Tocilizumab	12 (4.7)	33 (5.5)	0.661	23 (6.1)	22 (4.5)	0.300
Belimumab	13 (5.1)	17 (2.8)	0.093	16 (4.3)	14 (2.9)	0.277
Secukinumab	2 (0.8)	26 (4.3)	0.006	7 (1.9)	21 (4.3)	0.043
Rituximab	11 (4.3)	8 (1.3)	0.006	15 (4.0)	4 (0.8)	0.002
Ustekinumab	1 (0.4)	4 (0.7)	>0.999	2 (0.5)	3 (0.6)	0.869

Results are expressed as median (IQR) and *n* (%). Continuous data were compared using the Mann–Whitney *U*-test, and categorical variables with the chi-square or Fisher's exact test, as appropriate, always as two-sided analyses. SC defined as positive serology (IgG titer ≥15 AU ml^{-1}) for anti-SARS-CoV-2 S1/S2 IgG antibodies after vaccination (Indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG). Positivity for NAb defined as neutralizing activity ≥30% (cPass sVNT Kit).

patients with ARD have an increased risk of hospitalization for severe COVID-19 (refs. [21,24]). In this large prospective study of an inactivated SARS-CoV-2 vaccine in patients with ARD, CoronaVac demonstrated a good safety profile with no serious/moderate AEs related to the vaccine. The vaccine was immunogenic in patients with ARD, but at lower levels when compared to the CG. Controlling the groups for age was essential, since SC may be lower in the older population[10], and this differentiates the current trial from earlier studies[15–18].

We prospectively included a large population of patients with ARD representing eight systemic diseases fulfilling their respective classification criteria, and followed all participants with scheduled face-to-face appointments, telephone, smartphone instant messaging and email contacts, which allowed a more precise monitoring of vaccine-induced AEs in this population. Tolerance and safety are a relevant concern for patients with ARD, since they have an intrinsic risk for thrombosis[28], a rare complication reported for some of the new COVID-19 vaccines[29], and autoimmune/autoinflammatory manifestations, a problem with adjuvanted vaccines in this already predisposed population[30]. Similar to previous results from CoronaVac trials in healthy populations[31], most vaccine-related AEs were mild with pain at the injection site being the most frequently reported. Interestingly, vaccine-related AEs, particularly systemic symptoms, were much less frequent in both ARD and CG than those reported with mRNA vaccines[32,33]. These data confirm the previously reported safety profile of CoronaVac[11], and extend this finding to a large group of immunocompromised patients. Data on disease activity were not available due to the study design, with approximately 93% of participants vaccinated in a single center over 2 days, and therefore the influence of this factor on CoronaVac immunogenicity remains to be determined. The lack of assessment of vaccine T cell responses was another limitation of the present study[34,35].

The exclusion of seropositive participants and those with COVID-19 during the study period allowed a more accurate evaluation of the immunogenicity of CoronaVac. In addition, there was no difference in blood sample collection timing between the two groups because most participants received vaccine in the same timeframe, precluding the possible confounding nonlinear relationship between the elapsed time and the vaccine. We observed lower CoronaVac immunogenicity in patients with ARD, although within the immunologic response standards (SC rates and GMT) established by the European Medicine Agency and the Food and Drugs Administration recommendations for Emergency Use Authorization of pandemic vaccines[36,37]. The 70% SC rate was comparable to that obtained against the pandemic influenza A /H1N1 inactivated vaccine (approximately 63%)[27], but lower than those reported for the SARS-CoV-2 mRNA vaccine in a very small ARD population[17] and in a study with patients predominantly using cytokine inhibitors and with limited representation of systemic diseases[16]. There was a substantial increase in immune response parameters, including anti-SARS-CoV-2 IgG titers and SC and NAb positivity rates, only after the second dose, reinforcing the importance of the full vaccination schedule for optimal vaccine immunogenicity, particularly in the ARD group. Similar to the anti-SARS-CoV-2 IgG antibody response, the frequency of mean inhibitory neutralizing activity against SARS-CoV-2 (56.4%) was reduced compared to controls and that reported after SARS-CoV-2 mRNA vaccination[15,16]. Again, the second dose was essential to achieving the maximum response for both groups, with a lower neutralization activity in ARD than in CG after the two vaccine doses. A recent report including 53 patients with RA who had received mRNA vaccines also emphasized the importance of a second dose to improve immunogenicity[38].

The profile of tertiary hospital patients evaluated in this trial, with a high frequency being treated with immunosuppressive/glucocorticoid/biological therapies, probably contributed to the reduced humoral response observed in the ARD group. In fact, 63% were on immunosuppressive therapy and more than one-third on prednisone and biologics. Of note, these three groups of drugs were identified as independent variables that negatively impact both anti-SARS-CoV-2 IgG and neutralizing antibodies following vaccination. Among the immunosuppressive drugs, methotrexate and mycophenolate mofetil had the greatest negative impact on immunogenicity whereas abatacept and rituximab were the most negative among those treated with biologics. This finding is in line with other studies in patients with ARD and on other COVID vaccines[15,17,18,39] although these earlier reports did not control for age, which may limit the strength of the conclusions that can be drawn regarding the impact of these drugs[18]. Specifically for CoronaVac, these data added new information since another small trial found rituximab to be the only drug associated with low seropositivity after complete vaccination in immunocompromised patients[19]. We also found a detrimental effect of TNFi therapy solely on anti-S1/S2 IgG response, contrasting with a recent study of patients with ARD[16]. However, our findings require further investigation since most patients with CIA under TNFi were also being treated with methotrexate, which itself was associated with reduced humoral responses in the present trial.

Although not the main objective of this study, these data also provide preliminary evidence of the short-term efficacy of CoronaVac in prevention of symptomatic COVID-19 cases. An extension period of observation (up to 12 months) for incident cases is already in progress. Importantly, the majority of patients with ARD and CG were all vaccinated at the same epidemiological week over a 2-day period, providing a unique setting of comparable influence of the ongoing local SARS-CoV-2 infection rates. Remarkably, the 45% increase in COVID-19 cases in Sao Paulo occurred from mid-March through to the end of April, coinciding with the study period between D40 and D79 (>10 days after the second dose)[40]. In this 40-day interval in which vaccine immunity is already expected, the frequency of COVID-19 cases was notably lower than in the previous 40 days after the first vaccination (D0–D39). The unanticipated overall similar frequency of SARS-CoV-2 infection in patients with ARD, a known vulnerable immunosuppressed population, compared to CG during the study period may be explained by the higher adherence to social isolation and lower household contact with infected people, as well as by reduced use of public transportation among patients. It may also be related to high exposure due to the professions of the majority of CG. The small number of new RT–PCR-confirmed COVID-19 cases during the observation period hampers, however, a definitive conclusion on the role of vaccine efficacy. The Gamma variant was the dominant strain amongst incident cases, in line with the virologic surveillance in the region, where Gamma represented 90% of all sequenced samples in the state in late April 2021 followed by Alpha and Beta as the other VOC[41].

In conclusion, this study provides evidence of safety and reduced, but acceptable, short-term immunogenicity of an inactivated SARS-CoV-2 vaccine in the ARD population. The impact of this diminished humoral response on long-term vaccine effectiveness is already ongoing, and it will also shed light on the persistence of CoronaVac-elicited immune responses and the need for a vaccine booster.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-021-01469-5.

Received: 22 May 2021; Accepted: 15 July 2021;
Published online: 30 July 2021

References

1. WHO Coronavirus (COVID-19) Dashboard with vaccination data. *WHO* https://covid19.who.int (2021).
2. de Souza, W. M. et al. Epidemiological and clinical characteristics of the COVID-19 epidemic in Brazil. *Nat. Hum. Behav.* **4**, 856–865 (2020).
3. Faria, N. R. et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science* **372**, 815–821 (2021).
4. Coutinho, R. M. et al. Model-based estimation of transmissibility and reinfection of SARS-CoV-2 P. 1 variant. Preprint at https://www.medrxiv.org/content/10.1101/2021.03.03.21252706v (2021).
5. Funk, T. et al. Characteristics of SARS-CoV-2 variants of concern B.1.1.7, B.1.351 or P.1: data from seven EU/EEA countries, weeks 38/2020 to 10/2021. *Eurosurveillance* **26**, 2100348 (2021).
6. Coronavírus Brasil. Painel coronavírus. *Ministério da Saúde* https://covid.saude.gov.br/ (2021).
7. Bueno, S. M. et al. Interim report: safety and immunogenicity of an inactivated vaccine against SARS-CoV-2 in healthy Chilean adults in a phase 3 clinical trial. Preprint at https://www.medrxiv.org/content/10.1101/2021.03.31.21254494v1 (2021).
8. Dizeres de texto de bula – Professional da Saude. *Instituto Batantan* https://vacinacovid.butantan.gov.br/assets/arquivos/Bulas_Anvisa/2021.04.23 - Bula profissional da saúde.pdf (2021).
9. Dejnirattisai, W. et al. Antibody evasion by the P.1 strain of SARS-CoV-2. *Cell* **184**, 2939–2954 (2021).
10. Palacios, R. et al. Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in Brazil: the PROFISCOV Study. Preprint at https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3822780 (2021).
11. Zhang, M.-X. et al. Safety of an inactivated SARS-CoV-2 vaccine among healthcare workers in China. *Expert Rev. Vaccines* https://www.tandfonline.com/doi/full/10.1080/14760584.2021 (2021).
12. Harpaz, R., Dahl, R. M. & Dooling, K. L. Prevalence of immunosuppression among US adults, 2013. *JAMA* **316**, 2547–2548 (2016).
13. Boyarsky, B. J. et al. Immunogenicity of a single dose of SARS-CoV-2 messenger RNA vaccine in solid organ transplant recipients. *J. Am. Med. Assoc.* **325**, 1784–1786 (2021).

14. Boyarsky, B. J. et al. Antibody response to 2-dose SARS-CoV-2 mRNA vaccine series in solid organ transplant recipients. *J. Am. Med. Assoc.* **325**, 2204–2206 (2021).
15. Geisen, U. M. et al. Immunogenicity and safety of anti-SARS-CoV-2 mRNA vaccines in patients with chronic inflammatory conditions and immunosuppressive therapy in a monocentric cohort. *Ann. Rheum. Dis.* https://ard.bmj.com/content/early/2021/05/11/annrheumdis-2021-220272 (2021).
16. Simon, D. et al. SARS-CoV-2 vaccination responses in untreated, conventionally treated and anticytokine-treated patients with immune-mediated inflammatory diseases. *Ann. Rheum. Dis.* https://ard.bmj.com/content/early/2021/05/05/annrheumdis-2021-220461 (2021).
17. Boyarsky, B. J. et al. Antibody response to a single dose of SARS-CoV-2 mRNA vaccine in patients with rheumatic and musculoskeletal diseases. *Ann. Rheum. Dis.* https://ard.bmj.com/content/80/8/1098 (2021).
18. Furer, V. et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in adult patients with autoimmune inflammatory rheumatic diseases and in the general population: a multicentre study. *Ann. Rheum. Dis.* https://ard.bmj.com/content/early/2021/06/13/annrheumdis-2021-220647 (2021).
19. Seyahi, E. et al. Antibody response to inactivated COVID-19 vaccine (CoronaVac) in immune-mediated diseases: a controlled study among hospital workers and elderly. *Rheumatol. Int.* **41**, 1429–1440 (2021).
20. Grainger, R., Machado, P. M. & Robinson, P. C. Novel coronavirus disease-2019 (COVID-19) in people with rheumatic disease: epidemiology and outcomes. *Best Pract. Res. Clin. Rheumatol.* **35**, 101657 (2021).
21. Gianfrancesco, M. et al. Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Ann. Rheum. Dis.* **79**, 859–866 (2020).
22. Pablos, J. et al. Clinical outcomes of patients with COVID-19 and chronic inflammatory and autoimmune rheumatic diseases: a multicentric matched-cohort study. *Ann. Rheum. Dis.* **79**, 1544–1549 (2020).
23. Mikuls, T. R. et al. American College of Rheumatology Guidance for the Management of Rheumatic Disease in Adult Patients During the COVID-19 Pandemic: Version 3. *Arthritis Rheumatol.* **73**, e1–e12 (2021).
24. Strangfeld, A. et al. Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 Global Rheumatology Alliance physician-reported registry. *Ann. Rheum. Dis.* **80**, 930–942 (2021).
25. Avanzato, V. A. et al. Case study: prolonged infectious SARS-CoV-2 shedding from an asymptomatic immunocompromised individual with cancer. *Cell* **183**, 1901–1912 (2020).
26. Choi, B. et al. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. *N. Engl. J. Med.* **383**, 2291–2293 (2020).
27. Saad, C. G. S. et al. Immunogenicity and safety of the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune rheumatic diseases. *Ann. Rheum. Dis.* **70**, 1068–1073 (2011).
28. Lee, J. J. & Pope, J. E. A meta-analysis of the risk of venous thromboembolism in inflammatory rheumatic diseases. *Arthritis Res. Ther.* **16**, 435 (2014).
29. Hunter, P. R. Thrombosis after covid-19 vaccination. *Br. Med. J.* **16**, n958 (2021).
30. Shoenfeld, Y. & Agmon-Levin, N. 'ASIA' – autoimmune/inflammatory syndrome induced by adjuvants. *J. Autoimmun.* **36**, 4–8 (2011).
31. Zhang, Y. et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect. Dis.* **21**, 181–192 (2021).
32. Mulligan, M. J. et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* **586**, 589–593 (2020).
33. Baden, L. R. et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N. Engl. J. Med.* **384**, 403–416 (2021).
34. Barrett, J. R. et al. Phase 1/2 trial of SARS-CoV-2 vaccine ChAdOx1 nCoV-19 with a booster dose induces multifunctional antibody responses. *Nat. Med.* **27**, 279–288 (2021).
35. Folegatti, P. M. et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* **396**, 467–478 (2020).
36. Committee for Medicinal Products for Human Use (CHMP). *Guideline on Risk Management Systems for Medicinal Products for Human Use* (European Medicines Agency, 2018); https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-clinical-evaluation-vaccines-revision-1_en.pdf
37. *Guidance for Industry: Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines* (US Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research, 2007); https://www.fda.gov/files/vaccines,%20blood%20&%20biologics/published/Guidance-for-Industry–Clinical-Data-Needed-to-Support-the-Licensure-of-Pandemic-Influenza-Vaccines.pdf
38. Rubbert-Roth, A. et al. Anti-SARS-CoV-2 mRNA vaccine in patients with rheumatoid arthritis. *Lancet Rheumatol.* **3**, e470–e472 (2021).
39. Spiera R., Jinich S., Jannat-Khah D. Rituximab, but not other antirheumatic therapies, is associated with impaired serological response to SARS- CoV-2 vaccination in patients with rheumatic diseases. *Ann. Rheum. Dis.* https://ard.bmj.com/content/early/2021/05/10/annrheumdis-2021-220604 (2021).
40. Coronavírus – Boletim Completo. *Governo do Estado de São Paulo* https://www.seade.gov.br/coronavirus/# (2020).
41. Notícias Coronavírus: Variante P1 já responde por 90% das amostras em SP. *saopaulo.sp.gov.br* https://www.saopaulo.sp.gov.br/noticias-coronavirus/governo-de-sp-conclui-estudo-sobre-variantes-do-novo-coronavirus-no-estado/ (2021).

Methods

Ethics statement. The protocol was conducted according to the Declaration of Helsinki and local regulations, and approved by the National and Institutional Ethical Committee of Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil (no. CAAE: 42566621.0.0000.0068). Written informed consent was obtained from all participants before enrollment, including an agreement for sharing of source data following publication of this manuscript, with indirect identifiers. There was no participant compensation.

Study design. This phase 4 prospective controlled clinical trial (CoronavRheum clinicaltrials.gov, no. NCT04754698) was conducted at a single tertiary center in Brazil.

Patients and controls. Patients with ARD and ≥18 years of age from the Outpatient Rheumatology Clinics at our center were included, with the following diagnoses: RA[42], SLE[43], axSpA[44], PsA[45], primary vasculitis[46,47], pSSj[48], SSc[49], IIM[50] and PAPS[51].

After confirmation of participation by patients with ARD, CG were invited, with frequency matching by age (up to ±5 years difference) and sex, using an Excel program for random selection of participants (one control/five patients). None of these were previously vaccinated in the hospital's regular campaign. ARD diagnosis, use of immunosuppressive drugs and HIV infection were exclusion criteria for CG, whereas other well-controlled medical conditions were allowed in the CG group (Extended Data Fig. 1). None of the patients included in this analysis held medications to improve vaccine response.

Overall exclusion criteria were: history of anaphylactic response to vaccine components; acute febrile illness or symptoms compatible with COVID-19 at vaccination; Guillain–Barré syndrome; decompensated heart failure (class III or IV); demyelinating disease; previous vaccination with any SARS-CoV-2 vaccine; history of live virus vaccine up to 4 weeks previously; inactivated viral vaccine up to 2 weeks previously; history of having received blood products up to 6 months before the study; individuals who did not agree to participate in the study; hospitalized patients; and prevaccination positive COVID-19 serology and/or NAb (for immunogenicity analysis) (Extended Data Fig. 1).

After receiving the first vaccine dose, participants with RT–PCR-confirmed COVID-19 were excluded from the immunogenicity analysis but included in the evaluation of incident cases.

Vaccination protocol. The vaccination protocol for patients with ARD and GC consisted of a two-dose schedule of the COVID-19 vaccine. The first dose (with blood collection) was given for most participants on 9–10 February 2021 (D0), the second dose (with blood collection) on 9–10 March 2021 (D28) and a final blood collection on 19 April 2021 (D69) at the Hospital Convention Center. Incident COVID-19 cases were assessed for a further 10 days until D79. This protocol was delayed by 4 weeks for participants with incident COVID-19 during the study. Ready-to-use syringes loaded with CoronaVac (Sinovac Life Sciences, batch no. 20200412), consisting of 3 μg in 0.5 ml of β-propiolactone-inactivated SARS-CoV-2 (derived from the CN02 strain of SARS-CoV-2 grown in African green monkey kidney cells—Vero 25 cells) with aluminum hydroxide as an adjuvant, were administered intramuscularly in the deltoid area.

Primary and secondary outcomes. The primary outcome was humoral immunogenicity assessed by two coprimary endpoints: the presence of anti-S1/S2 SARS-CoV-2 IgG and the presence of NAb 6 weeks after the second vaccine dose (D69).

Secondary immunogenicity outcomes were: anti-S1/S2 IgG seroconversion and the presence of NAb at D28 (after vaccine first dose); geometric mean titers of anti-S1/S2 IgG and their factor increase in GMT (FI-GMT) at D28 and D69; and median (IQR) neutralizing activity of NAb at D28 and D69.

A further secondary outcome was safety related to the vaccine doses. Additionally, factors associated with anti-SARS-CoV-2 IgG SC and NAb positivity and incident COVID-19 case evaluation were exploratory outcomes.

Samples for immunogenicity evaluation. To assess these outcomes, blood samples (20 ml) from all participants were obtained at D0 (baseline, immediately before first vaccine dose), D28 (immediately before the second dose) and D69 (6 weeks after the second dose). Sera were stored in a freezer at −70 °C.

Anti-SARS-CoV-2 S1/S2 IgG antibodies. A chemiluminescent immunoassay was used to measure human IgG antibodies against proteins S1 and S2 in the receptor-binding domain (RBD) (Indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG, DiaSorin). SC rate was defined as positive serology (≥15.0 UA ml^{-1}) after vaccination, taking into consideration that only patients with prevaccination negative serology were included. GMT and 95% CIs of these antibodies were also calculated at all time points, attributing the value of 1.9 UA ml^{-1} (half of the lower limit of quantification, 3.8 UA ml^{-1}) to undetectable levels (<3.8 UA ml^{-1}). FI-GMT is the ratio of GMT after vaccination to that before, with growth measured in titers. These values are also presented and compared as geometric means and 95% CIs.

SARS-CoV-2 cPass virus NAb. The SARS-CoV-2 sVNT Kit (GenScript) was utilized according to the manufacturer's instructions. This analysis detects circulating NAb against SARS-CoV-2 that block the interaction between the RBD of the viral Spike glycoprotein with the angiotensin-converting enzyme 2 cell surface receptor. Tests were performed on ETI-MAX-3000 equipment (DiaSorin). Samples were classified as either "positive" (inhibition ≥30%) or "negative" (inhibition <30%), as suggested by the manufacturer[52]. The frequency of positive samples was calculated at all time points. Medians (IQR) of the percentage of neutralizing activity, for positive samples only, were calculated at all time points.

Vaccine AEs and incident cases of COVID-19. Safety was rigorously followed by the National Research Ethics Council, and all serious AEs were classified as either vaccine related or not related. In addition an independent Data Safety Monitoring Board, comprising vaccine-prominent experts, periodically reviewed and evaluated the study protocol. Patients and control groups were advised to report any side effects of the vaccine; to this end, they received on D0 (first dose) and D28 (second dose) a standardized diary for recording of local and systemic manifestations. Local manifestations included local pain, erythema, swelling, bruising, pruritus and induration at the vaccine site. Systemic reactions included fever, malaise, somnolence, lack of appetite, nausea, vomiting, diarrhea, abdominal pain, vertigo, tremor, headache, fatigue, myalgia, muscle weakness, arthralgia, back pain, cough, sneezing, coryza, stuffy nose, sore throat, shortness of breath, conjunctivitis, pruritus and skin rash. Vaccine AE severity was defined according to the WHO definition[53].

Environmental factors associated with high risk of exposure to SARS-CoV-2 were recorded from all participants, including adherence to social isolation, number of people living in the same house, household contact with infected people and use of public transportation.

Additionally, to evaluate incident COVID-19 cases (exploratory outcome), all patients with ARD and controls were instructed to communicate any manifestation associated or not with COVID-19 by telephone, smartphone instant messaging or email. Our medical team was divided to provide a proper follow-up for the assigned group of patients/controls including the need for medical care, hospitalizations, severity of infections, sick days and treatment. Participants with suspicion of COVID-19 were instructed to seek medical care near their residence and, if recommended, to come to our tertiary hospital to undergo a RT–PCR test for SARS-CoV-2 or make an in-person visit. If tertiary care was required, the participant was transferred to a referenced hospital. The standardized diary of AEs was carefully reviewed with each participant on the day of the second dose (D28) and at the last visit (D69). COVID-19 incident cases were followed for 40 days (from D0 to 10 days after the second dose (D39)) and thereafter for the following 40 days (from D40 to D79).

Study data were collected and managed using REDCap electronic data capture tools (10.5.0, 2021 Vanderbilt University) hosted at our Institution[54,55].

RT–PCR for SARS-CoV-2 and analysis of VOC. Clinical samples for SARS-CoV-2 RT–PCR consisted of naso- and oropharyngeal swabs, using a laboratory-developed test[56]. All participants with positive test results were invited to collect samples at our hospital, and these materials were further analyzed for VOC. RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions. For rapid access of VOC, we performed two real-time PCR protocols in parallel. Romano et al.[57] used two sets of probes to detect NSP6 Δ 106–108, which encodes a protein that participates in the viral replication process and allows the differentiation of ancestral variants from Alpha, Beta and Gamma VOC. The protocol of Vogels et al. uses a multiplex quantitative RT–PCR (RT–qPCR) assay that targets three regions (N1, ORF1a Δ3675–3677 and Spike Δ69–70 primer) and facilitates differentiation of Alpha VOC from Beta and Gama VOC, and from ancestral variants[58]. To confirm the results, we sequenced the virus using a combination of targeted multiplex PCR amplification and a portable nanopore sequencing MinION platform (Oxford Nanopore Technologies)[3,58]. In brief, complementary DNA was synthesized with random hexamers and the Protoscript II First Strand cDNA synthesis Kit (New England Biolabs). Whole-genome multiplex PCR amplification was then conducted using the ARTIC network SARS-CoV-2 V3 primer scheme. Multiplex PCR products were purified using AmpureXP beads (Beckman Coulter), and quantification was carried out using the Qubit dsDNA High Sensitivity assay on the Qubit 3.0 (Life Technologies). Samples were then normalized (10 ng per sample), DNA fragments were barcoded using the EXP-NBD104 (refs. [59,60]) and EXP-NBD114 (ref. [61]) Native Barcoding Kits (Oxford Nanopore Technologies) and pooled. Sequencing adapter ligation was performed using the SQK-LSK 109 Kit (Oxford Nanopore Technologies). Sequencing libraries were loaded onto an R9.4.1 flow-cell (Oxford NanoporeTechnologies) and sequenced using MinKNOW v.20.10.3 (Oxford Nanopore Technologies).

Symptomatic participants who were unable to come to our center to collect the RT–PCR kit were instructed to go to an independent laboratory near their home.

Statistical analysis. Sample size calculation was based on the previous 15% reduction in SC rate after first vaccination with the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of patients with ARD[36]. In expectation of

SC rates of 63% in the ARD patient cohort and 78% in the control group, and considering an alpha error of 5% and power of 80% in a 5/1 ratio to include more patients with ARD, the minimum sample required would be 445 patients with ARD and 89 healthy subjects, sex controlled and of similar age. In expectation of a higher SC rate of 98% for this vaccine[28], such sample size had a power >99% to detect a 15% reduction in SC of patients with ARD. Due to the peak of the ongoing pandemic in Brazil during the vaccination period, we invited additional patients and controls, expecting a high incidence of previously infected people and a high rate of infection.

Categorical variables are presented as number (percentage) and compared using the chi-square or Fisher's exact test, as appropriate. Only for patients with ARD, multivariate logistic regression analyses were performed using as dependent variables SC or the presence of NAb at D69 (primary endpoints), and as independent variables those with $P < 0.2$ in each univariate analysis.

Continuous general data are presented as medians (IQR) and compared using the Mann–Whitney *U*-test for intergroup comparison. Continuous data regarding anti-S1/S2 serology titers are presented as geometric means (95% CI); their comparisons were performed using repeated-measures analysis of variance (ANOVA) with two factors (two groups (ARD and CG) at three time points (D0, D28 and D69)), followed by Bonferroni's multiple comparisons in ln-transformed data.

Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences, v.20.0 (IBM-SPSS for Windows 20.0).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All background information on controls and clinical information for patients with ARD in this study are included in the Source data provided with this paper (https://figshare.com/s/0a8921e7422a4fb8436f). Requests for sera sharing will need approval from the Hospital das Clinicas da Universidade de Sao Paulo´s review board and the National Research Ethics Council and a Material Transfer Agreement, which typically requires about 1 month. The SARS-CoV-2 sequences are available on GISAID (http://www.gisaid.org) (nos. EPI_ISL_2894869–2894885). An account (free registration) on GISAID is needed to obtain access to sequences. Additional correspondence and requests for materials should be addressed to the corresponding author (E.B.).

References

42. Aletaha, D. et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum.* **62**, 2569–2581 (2010).
43. Petri, M. et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum.* **64**, 2677–2686 (2012).
44. Rudwaleit, M. et al. The development of Assessment of SpondyloArthritis International Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann. Rheum. Dis.* **68**, 777–783 (2009).
45. Tillett, W. et al. The ClASsification for Psoriatic ARthritis (CASPAR) Criteria – a retrospective feasibility, sensitivity, and specificity study. *J. Rheumatol.* **39**, 154–156 (2012).
46. Arend, W. P. et al. The American College of Rheumatology 1990 criteria for the classification of takayasu arteritis. *Arthritis Rheum.* **33**, 1129–1134 (2010).
47. Leavitt, R. Y. et al. The American College of Rheumatology 1990 criteria for the classification of Wegener's granulomatosis. *Arthritis Rheum.* **33**, 1101–1107 (2010).
48. Vitali, C. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann. Rheum. Dis.* **61**, 554–558 (2002).
49. van den Hoogen, F. et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Ann. Rheum. Dis.* **72**, 1747–1755 (2013).
50. Lundberg, I. E. et al. EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann. Rheum. Dis.* **76**, 1955–1964 (2017).
51. Miyakis, S. et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J. Thromb. Haemost.* **4**, 295–306 (2006).
52. Taylor, S. C. et al. A new SARS-CoV-2 dual-purpose serology test: highly accurate infection tracing and neutralizing antibody response detection. *J. Clin. Microbiol.* **59**, 4 (2021).
53. *WHO Draft Guidelines for Adverse Event Reporting and Learning Systems* (WHO, 2021); https://apps.who.int/iris/bitstream/handle/10665/69797/WHO-EIP-SPO-QPS-05.3-eng.pdf?sequence=1&isAllowed=y
54. Harris, P. A. et al. The REDCap consortium: building an international community of software platform partners. *J. Biomed. Inform.* **95**, 103208 (2019).
55. Harris, P. A. et al. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J. Biomed. Inform.* **42**, 377–381 (2009).
56. Corman, V. M. et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT–PCR. *Eurosurveillance* **25**, 2000045 (2020).
57. Romano, C. M. et al. Real-time PCR protocol to screen for SARS-COV-2 variants of concern (B. 1.1. 7, P. 1 and B. 1.1. 35). *protocols.io* https://www.protocols.io/view/real-time-pcr-protocol-to-screen-for-sars-cov-2-va-bszbnf2n (2021).
58. Vogels, C. B. F. et al. Multiplex qPCR discriminates variants of concern to enhance global surveillance of SARS-CoV-2. *PLoS Biol.* **19**, e3001236 (2021).
59. Hallal, P. C. et al. SARS-CoV-2 antibody prevalence in Brazil: Results from two successive nationwide serological household surveys. *Lancet Glob. Health* **8**, e1390–e1398 (2020).
60. Buss, L. F. et al. Three-quarters attack rate of SARS-CoV-2 in the Brazilian Amazon during a largely unmitigated epidemic. *Science* **371**, 288–292 (2021).
61. Rambaut, A. et al. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. *Virological.org* https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563 (2020).

Acknowledgements

We thank the contribution of the Central Laboratory Division, Registry Division, Security Division, IT Division, Superintendence, Pharmacy Division and Vaccination Center for their technical support. We also thank the volunteers for participating in the three in-person visits of the protocol and for handling the biological material, and those responsible for the follow-up of all participants. This study was sponsored by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (no. 2015/03756–4 to N.E.A., S.G.P., C.A.S. and E.B.; no. 2017/14352-7 to T.P., no. 2018/09937-9 to V.A.O.M., no. 2020/11677-5 to G.B.H.D.; and no. 2019/21173-7 to C.T.R.) and from Conselho Nacional de Desenvolvimento Científico e Tecnológico (no. 305242/2019-9 to E.B.; no. 304984/2020-5 to C.A.S.; no. 305556/2017-7 to R.M.R.P.; and no. 303379/2018-9 to S.K.S.). Instituto Butantan supplied the study product and had no other role in the trial.

Author contributions

A.C.M.-R., N.E.A., C.G.S., E.F.N.Y., T.P., S.G.P., E.G.K. and E.B. conceived and designed the study, participated in data collection and analysis and supervised clinical data management, writing of the manuscript and revision of the manuscript. S.G.R.F. and P.T.R. organized and supervised blood collection and vaccination. A.J.S.D. and L.A. supervised serum processing, SARS-CoV-2-specific antibody ELISA/neutralization assays and SARS-CoV-2 RT–PCR. A.C.M.-R., N.E.A., C.G.S., E.F.N.Y., T.P., S.G.P., E.B, S.R.G.F., P.T.R., R.M.R.P., S.K.S., D.C.O.A., P.D.S.-B., C.T.R., G.B.H.D., V.A.O.M. and C.A.S. collected epidemiological and clinical data and assisted with the identification of SARS-CoV-2 infection and follow-up of patients. M.H.L. organized and supervised the vaccination protocol. E.C.S. performed SARS-CoV-2 genotyping of positive RT–qPCR samples and screening of variants of concern. All authors helped to edit the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

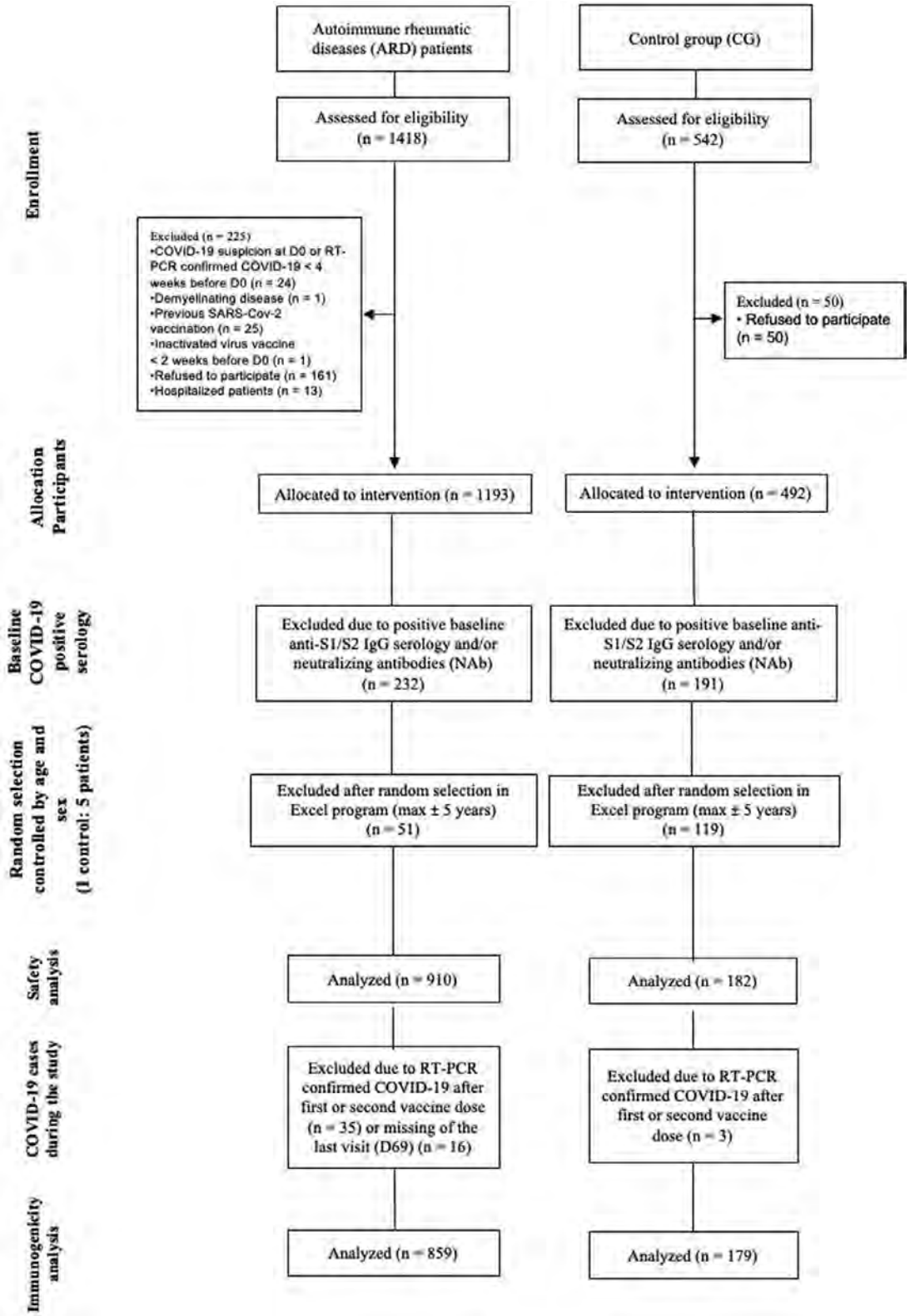
Extended data is available for this paper at https://doi.org/10.1038/s41591-021-01469-5.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-021-01469-5.

Correspondence and requests for materials should be addressed to E.B.

Peer review information: *Nature Medicine* thanks Dawn Teare, Philip Robinson and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Saheli Sadanand was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

Reprints and permissions information is available at www.nature.com/reprints.



Extended Data Fig. 1 | Trial Design. The diagram depicts the enrollment and analysis of participants in the ARD and CG groups. Reasons for exclusions are provided.

nature research

Corresponding author(s): Eloisa Bonfa

Last updated by author(s): Jul 15, 2021

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	The analyzed data were extracted from the patients' electronic medical records (PRONTMED) and Study data were collected and managed using REDCap electronic data capture tools (10.5.0 - © 2021 Vanderbilt University) hosted at our Institution. Data collection for ELISA was performed using Indirect ELISA, LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy. Neutralizing antibodies were performed on the ETI-MAX-3000 equipment (DiaSorin, Italy). No custom software codes have been developed.
Data analysis	All statistical analyses were performed using Statistical Package for the Social Sciences version 20.0 (IBM-SPSS for Windows. 20.0. Chicago, IL, USA)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All the background information on controls and clinical information for the patients with ARD in this study are included in the Source Data provided with this paper (https://figshare.com/s/0a8921e7422a4fb8436f). Requests for sera sharing will need approval from the Hospital das Clinicas da Universidade de Sao Paulo´s review

board and the National Research Ethics Council and a Material Transfer Agreement, which typically requires about one month. The SARS-CoV-2 sequences are available on GISAID (http://www.gisaid.org) (EPI_ISL_2894869 to 2894885). An account (free registration) on GISAID is needed in order to obtain access to the sequences. Additional correspondence and requests for materials should be addressed to the corresponding author (E.B.).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size calculation was based on the previous 15% reduction of seroconversion rate after primo vaccination with the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of ARD patients35. Expecting seroconversion rates of 63% in the ARD patient's cohort and 78% in the control group, considering an alpha error of 5% and power of 80%, in 5 : 1 ratio in order to include more ARD patients, the minimum sample required would be 445 ARD patients and 89 healthy subjects, sex-matched and with similar ages.
Data exclusions	All safety and immunogenicity data were included in the study. No data were excluded from the analyses.
Replication	This is an ongoing human trial and therefore there was still no attempt of replication.
Randomization	This was an observational study with no randomized intervention. All participants (patients and controls) received the same vaccine, without experimental groups.
Blinding	This phase 4 prospective controlled observational study with no randomized intervention. All participants (patients and controls) received the same vaccine, without placebo group. Therefore, blinding was not performed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	Male and female individuals with autoimmune rheumatic disease and volunteers (control group) ≥ 18 anos. The 910 patients with ARD and 182 controls included in immunogenicioty analysis had comparable median ages [51 (40-60) vs. 50 (41-60) years, p=0.985] and female sex (76.9% vs. 76.9%, p>0.999). Three hundred and forty-eight (38.2%) patients were receiving ongoing treatment with prednisone, median dose 5 (5-10) mg/day, 573 (63.0%) were using immunosuppressive drugs [methotrexate (25.2%), leflunomide (14.3%), mycophenolate mofetil (13.1%), azathioprine (12%) and others less than 3% each] and 321 (35.3%) were under biologic therapy.
Recruitment	Autoimmune rheumatic disease (ARD) patients from the Outpatient Rheumatology Clinics at Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil. Control Group (CG) were invited, matching by gender and sex (up to ± 5 years differences). None of them were previously vaccinated in the hospital's regular campaign. Well-controlled medical conditions were allowed in the CG, except ARD, use of immunosuppressive drugs or HIV infection. Overall exclusion criteria were: history of anaphylactic response to vaccine components, acute febrile illness or symptoms compatible to COVID-19 at vaccination, Guillain-Barré syndrome, decompensated heart failure (class III or IV), demyelinating disease, previous vaccination with any SARS-Cov-2 vaccine, history of live virus vaccine up to four weeks before, virus vaccine inactivated up to two weeks before, history of having received blood products up to six months before the study, individuals

who did not accept to participate in the study, hospitalized patients, and pre-vaccination positive COVID-19 serology and/or neutralization antibodies.
All statistical analyses took into account the frequency matching, with exclusion of non-matched subjects. Immunogenitics analysis also excluded incident COVID-19 cases and patients who did not attend the final visit, composing the final sample of 859 patients with ARD and 179 CG. In the logistic regression model (Supplementary Table 2, only with patients with ARD), the age was included in the model using the cut-off > 60 years. This model intended to highlight the known importance of older age in vaccine response.

Ethics oversight

The protocol was conducted according to the Declaration of Helsinki and local regulations and approved by the National and Institutional Ethical Committee of Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil (CAAE: 42566621.0.0000.0068). Written informed consent was obtained from each participant before enrollment.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

ClinicalTrials.gov Identifier: NCT04754698

Study protocol

The protocolo has been submitted.The full trial can be assessed at ClinicalTrials.gov

Data collection

The study was conducted at a single tertiary center in Brazil. Enrollment and vaccination occurred in the same day for each participant. The first subject was enrolled and vaccinated on Feb 9th, 2021 and the last participant was enrolled and vaccinated on February 24th, 2021. The vaccination protocol for patients with ARD and controls consisted of a two-dose schedule of the COVID-19 vaccine. The first dose with blood collection was given for most of participants on February 9-10th 2021 (D0), the second dose with blood collection on March 9-10th 2021 (D28) and the last blood collection on April 19th 2021 (D69) at the Hospital Convention Center. Incident COVID-19 cases were assessed for another 10 days to D79.
During 2 consecutive days of the 2021 epidemiological week 6th, all ARD patients and CG received the 1st CoronaVac dose, repeated at a 2-dose schedule after 28 days. Blood samples were collected from all participants for quantitative serological testing for SARS-CoV-2. The primary outcome was seroconversion rate (SC) at 6 weeks after the 2nd dose. Geometric meantitles (GMT) and factor increase in GMT (FI-GMT) were also calculated. ARD patients and CG were evaluated using standardized vaccination and COVID-19 symptom diaries, 3 face-to-face visits, and 24-hs available phone, whatsapp and e-mail contact. Symptomatic cases were tested by RT-PCR for SARS-CoV-2 and a subgroup of positive samples were evaluated for the presence of variants of concerns (Gamma, Alpha and Beta lineages).

Outcomes

Immunogenicity and safety of the CoronaVac vaccine in ARDs patients.
Primary Outcome Measure: presence of ≥30% of neutralizing activity of SARS-CoV-2 antibodies and seroconversion rate of anti-SARS-Cov-2 IgG antibodies.
Secondary outcome: safety of CoronaVac in a large cohort of ARD patients compared with age- and sex-matched controls without these conditions. Incident symptomatic cases confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) for SARS-CoV-2 and the presence of variants of concerns (Gamma, Alpha and Beta lineages).

4.13. CoronaVac ajuda a melhorar imunidade em pacientes transplantados, afirma estudo da Unifesp e USP

Um estudo realizado por pesquisadores do Instituto Butantan, da Universidade Federal de São Paulo (Unifesp) e do Hemocentro de Ribeirão Preto da Universidade de São Paulo (USP) mostrou que 43% dos pacientes transplantados de rim geraram anticorpos contra a Covid-19 15 dias após receberem a segunda dose da CoronaVac (ou seja, apresentaram soroconversão). O resultado indica que a vacina do Butantan e da farmacêutica chinesa Sinovac tem desempenho nesse público levemente superior ao de dois outros imunizantes, que utilizam a tecnologia de RNA mensageiro, e geraram anticorpos em pouco mais de 30% dos casos, segundo estudos.

Esses dados mostram a importância da vacina também para todos os imunossuprimidos que, assim como os transplantados e as pessoas com doenças autoimunes, possuem maior dificuldade na defesa imunológica do organismo.

"Toda vacina é menos eficaz em quem é transplantado por causa do uso das medicações contra a rejeição ao transplante. Isso acontece com os imunizantes contra hepatite B, gripe, pneumonia, e também com a vacina contra o coronavírus", explica o principal autor do artigo e professor titular da área de transplantes da Escola Paulista de Medicina da Unifesp, José Medina.

O trabalho foi desenvolvido no Hospital do Rim e seus resultados preliminares foram divulgados em artigo na revista Transplantation, a principal publicação mundial da área de transplantes. A pesquisa foi realizada entre 20 e 28 de março de 2021 com 3.354 pacientes transplantados renais entre 30 e 69 anos, que haviam realizado o transplante há mais de 30 dias, não apresentavam caso anterior de Covid-19 e completaram o esquema vacinal de duas doses da CoronaVac com intervalo de 28 dias.

"Como o número de transplantados é muito pequeno dentro da população em geral, assim que a maioria das pessoas estiver vacinada cairá a circulação do coronavírus, protegendo também os transplantados", afirma Medina. As taxas de soroconversão entre os transplantados renais após a primeira e segunda doses da CoronaVac alertam para a necessidade de manutenção das medidas de proteção individual, como usar máscara, evitar aglomerações e higienizar sempre as mãos.

Os receptores de transplante renal foram incluídos no calendário nacional de vacinação contra a Covid-19 no público prioritário com comorbidades a partir de abril, em função das elevadas taxas de mortalidade associadas ao SARS-CoV-2 nessa população (de até 30%).

As conclusões do estudo da Unifesp se somam a outra pesquisa realizada pelo Hospital das Clínicas da USP, na qual 1000 pacientes com doenças reumatológicas (também imunossuprimidos) foram vacinados com a CoronaVac. A imunização gerou uma resposta imune moderada nos pacientes: o acompanhamento pré e pós vacina mostrou 33 casos de Covid-19 antes da vacinação e apenas seis casos após a imunização.

A eficácia da CoronaVac foi comprovada no Brasil por meio de um estudo com 13.060 voluntários, todos profissionais da saúde, população altamente exposta à Covid-19. Os resultados do estudo clínico de fase 3 demonstraram que a eficácia geral do imunizante pode chegar a 62,3% quando o intervalo entre a primeira e a segunda dose da vacina é de 21 a 28 dias. Os dados foram divulgados na plataforma de preprints da revista The Lancet e estão em processo de revisão por pares.

Publicado em: 9/07/2021

Transplantation Publish Ahead of Print

DOI: 10.1097/TP.0000000000003901

Clinical Impact, Reactogenicity, and Immunogenicity After the First CoronaVac Dose in Kidney Transplant Recipients

José Medina-Pestana, PhD,[1] Marina Pontello Cristelli, PhD,[1] Laila Almeida Viana, MD,[1] Renato Demarchi Foresto, MD,[1] Lucio R. Requião-Moura, PhD,[1] Helio Tedesco-Silva, PhD,[1] Dimas Tadeu Covas, PhD[2,3]

[1] Nephrology Division, Hospital do Rim, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil.

[2] Instituto Butantan, São Paulo, Brazil.

3 Center for Cell-based Therapy (CTC), Regional Blood Center of Ribeirão Preto, University of São Paulo, São Paulo, Brazil.

Funding: No funding

Disclosure: The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

Author contributions

J.M.-P., M.P.C., L.A.V., H.T.-S., and D.T.C. participated in the research design; J.M.-P., M.P.C., L.A.V., R.D.F., L.R.R.-M., and H.T.-S. participated in the writing of the paper; and M.P.C., L.A.V., and H.T.-S. participated in data analysis.

Corresponding author: Marina Pontello Cristelli Nephrology Division, Hospital do Rim, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil. Postal address: Borges Lagoa, 960, São Paulo, Brazil 04038-002 E-mail address: ninacristelli@yahoo.com.br Telephone number: +551155768000

Introduction

In phase-3 trial, inactivated SARS-CoV-2 vaccine (CoronaVac, Sinovac Life-Sciences, Beijing, China) was associated with 71.1% seroconversion at least 14-days after the 2nddose, showing 50.7% efficacy against symptomatic COVID-19 among healthcare workers.[1] Currently this vaccine has been approved for emergency use in 24 countries, including Brazil, where the national vaccination program was launched on 01/Jan/2021 following the age criterion.

Kidney transplant recipients have shown 20-30% COVID-19-associated fatality rates,[2] have been excluded in vaccine trials, and had no early priority for vaccination.

Therefore, this single-center, prospective, 12-month follow-up study was designed to assess clinical impact, reactogenicity, and immunogenicity of CoronaVac.

Materials and Methods

Between March 20th and 28th 2021, 3354 patients aged 30-69 years, >30 days of transplantation and no previous COVID-19 received standard two-dose schedule of CoronaVac (3µg each dose, 28 days apart). Patients were scheduled to receive the vaccine on 2 consecutive weekends, with approximately 800-900 patients per day, from 7 a.m. to 7 p.m. All communication resources were used to reach them within 3 weeks before the vaccination day (telephone call, SMS text messages, WhatsApp messages). Workstations were set up at the outpatient clinic and patients were admitted in groups of 30 persons to: a) obtain general information regarding COVID-19, the clinical study, the vaccine, and preventive measures; b) inform consent discussion and signature; c) registration of the patient in the electronic medical records of the institution; d) blood sampling for serology followed by vaccination, and a reminder of the scheduled second dose. All employees of the institution were invited to participate in the vaccination campaign, and more than 300 professionals volunteered for the activity, including students from different universities.

The study was approved by the local ethics committee, registered at ClinicalTrials.gov, NCT04801667, and all patients signed an informed consent-form. At day 28, a prespecified questionnaire was obtained to capture adverse reactions to the vaccine or newly diagnosed SARS-CoV-2 infection. Sample-size for the immunogenicity cohort (942 patients seronegative for IgG anti-SARS-CoV-2 before first dose) was calculated using the age distribution and seroconversion rate (71%) of the phase-3 study,[1] with 95%CI and an absolute error of 10%. Antibody response at day 28 was assessed using the AdviseDx SARS-CoV-2 IgG II assay (Abbot Laboratories, Il, USA). Values >50 arbitrary units (AUs)/mL were considered positive.[3]

Results

Characteristics and outcomes of the study population (n=3354) are in Table 1. They were predominantly male, median age of 52 (interquartile range, IQR 44-60) years, low prevalence of diabetes mellitus, and median time posttransplant of 7 (IQR 3 – 12) years. Seroprevalence of IgG anti-SARS-CoV-2 nucleocapsid protein at D0 was 3.6%, and these seropositive patients at the time of vaccine were excluded for the analysis of the antibody responses. Among the seronegative patients at D0, there were 1012 individuals randomly selected for the immunogenicity analysis. The other patients did not have any testing performed after the vaccination. After the first vaccine dose, 61 (1.8%) patients had COVID-19 confirmed by RT-PCR or antigen-test at a median time of 12 (IQR 8-16) days. Of them, 44 (72%) required hospitalization and 16 (26%) died 14-49 days after the first vaccine dose.

The most common adverse-reaction was local pain/tenderness (11%). Systemic symptoms occurred in 5% or less of the patients; no severe adverse reaction was observed. There was only one episode of acute cellular rejection (Banff IB) 6 days after vaccination in a patient with documented nonadherence that showed partial recovery of renal function after treatment with methylprednisone and anti-thymocyte globulin. Seroconversion 28 days after the 1st dose was 15.2% (95%CI 12.9%-17.5%), median IgG value of 477 AUs/mL (IQR 123-1705). Patients over 60-years and combined kidney-pancreas-transplants had lower seroconversion than those younger than 60-years and isolated kidney-transplants.

Discussion

The potential advantage of the traditional inactivated vaccines, the induction of a broader polyclonal immune response,[4] was not associated with a higher seroconversion rate compared to the newer RNA-based COVID-19 vaccines.[5] In this ongoing prospective study, there was no obvious clinical impact after the first dose, as demonstrated by the 26% lethality rate, similar to that of unvaccinated kidney transplant recipients.[2] CoronaVac vaccine was safe, but seroconversion after the first dose was low, similar to what was reported to the RNA-based vaccines. The elderly showed even lower rates of seroconversion. These findings support the need for maintaining individual protection measures, even after the 1st dose of the vaccine.

Acknowledgments

This work would not be possible without the provision of the vaccines, coordinated by Ricardo Palacios, MD, PhD and Roberta Piorelli, MD from Instituto Butantan, the volunteer work of almost 300 students and professionals of the Hospital do Rim and other medical institutions in São Paulo during vaccination, and the valuable contribution of our biochemist Elizabeth França Lucena, BA, who conducted all the laboratory analysis.

References

1. Palacios R, Batista AP, Albuquerque CSN, et al. Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in Brazil: the PROFISCOV study. *SSRN*. 2021. doi:10.2139/ssrn.3822780

2. Cristelli MP, Viana LA, Dantas MTC, et al. The full spectrum of COVID-19 development and recovery among kidney transplant recipients. *Transplantation*. 2021;105(7):1433-1444.

3. Abbott Laboratories. AdviseDx SARS-CoV-2 IgG II instructions for use, H18575R01. February 2021. Available at https://www.fda.gov/media/146371/download. Accessed March 1, 2021.

4. Galdiero M, Galdiero M, Folliero V, et al. SARS-CoV-2 vaccine development: where are we? *Eur Rev Med Pharmacol Sci*. 2021;25(6):2752-2784.

5. Boyarsky BJ, Werbel WA, Avery RK, et al. Immunogenicity of a single dose of SARS-CoV-2 messenger RNA vaccine in solid organ transplant recipients. *JAMA*. 2021;325(17):1784-1786.

Table 1. Baseline demographic characteristics, outcomes, adverse reactions, and immunogenicity of the first dose of CoronaVac in kidney transplant recipients.

Parameters	Overall (n = 3354)	Immunogenicity cohort (n = 942)	*P*	IgG (+) D28 (n = 143)	IgG (−) D28 (n = 799)	*P*
Demographic characteristics						
Median age, (IQR), y	52 (44–60)	50 (43–56)	<0.001	47 (41–54)	51 (43–57)	< 0.001
30–60 years, n (%)	2552 (76)	844 (90)		136 (95)	708 (89)	
Over 60 years, n (%)	802 (24)	98 (10)		7 (5)	91 (11)	
Male gender, n (%)	2008 (60)	544 (58)	0.269	76 (53)	468 (59)	0.556
Diabetes mellitus, n (%)	333 (10)	93 (10)	> 0.99	10 (7)	83 (10)	0.208
Organ, n (%)						
Kidney	3239 (96)	835 (89)	< 0.001	140 (98)	695 (87)	< 0.001
Simultaneous pancreas-kidney	115 (4)	107 (11)		3 (2)	104 (13)	
Median length of transplant, (IQR), y	7 (3–12)	6 (3–11)	<0.001	6 (3–11)	6 (3–11)	> 0.99
Maintenance immunosuppressive regimen, n (%)						
TAC-Pred-AZA	1002 (30)	282 (30)		34 (24)	248 (31)	
TAC-Pred-MPA	1396 (42)	402 (43)		66 (46)	336 (42)	
CSA-Pred-AZA	376 (11)	89 (9)	0.231	11 (8)	78 (10)	0.253
TAC-Pred-mTORi	306 (9)	102 (11)		18 (12)	84 (10)	
Other	274 (8)	67 (7)		14 (10)	53 (7)	
Outcomes						
COVID-19 diagnosis after the 1st dose, n (%)	61 (1.8)					
Median age, (IQR), y	53 (47–59)					
Time from 1st dose to COVID-19, n (%)						
≤7 d	13 (21)					
8–14 d	23 (38)					
>14 d	25 (41)					
Need for hospitalization, n (%)	44 (72)					
Need for intensive care, n (%)	27 (44)					
Lethality from COVID-19, n (%)	16 (26)					
Adverse reactions to the vaccine, n (%) (n = 3274)						

Local pain or tenderness	378 (11)			
Headache	178 (5)			
Myalgia	160 (5)			
Runny nose	113 (3)			
Diarrhea	93 (3)			
Sore throat	65 (2)			
Fever	39 (1)			
Serologic status before vaccination, n (%)				
Negative	3182 (95)	942	-	-
Positive	122 (4)	0	-	-
Indeterminate	50 (1)	0	-	-
Serologic status after the 1st dose, n (%)				
Negative (<50 AUs/mL)	-	799 (85)		
Positive[a]	-	143 (15); 95% CI, 13%–17%		
30–60 y, n (%)		134 (16); 95% CI, 14%–19%		
>60 y, n (%)		9 (8); 95% CI, 3%–13%		

[a]$P = 0.026$ for comparison between the two-age range.

AZA, azathioprine; COVID-19, coronavirus disease 2019; CSA, cyclosporine; IgG, immunoglobulin G; IQR, interquartile range; MPA, mycophenolate; mTORi, mammalian target of rapamycin inhibitors; Pred, prednisone; TAC, tacrolimus.

5. É eficaz em idosos

5.1. CoronaVac é segura para idosos e taxa de eventos adversos é baixa, mostra pesquisa

Um estudo realizado em Hong Kong para avaliar o risco de eventos adversos em idosos imunizados com a CoronaVac comprovou mais uma vez a segurança da vacina, com ocorrência de 79,9 eventos adversos leves a cada 100 mil idosos vacinados. O trabalho foi publicado na revista The Lancet Health Longevity e conduzido por pesquisadores da Faculdade de Medicina da Universidade de Hong Kong.

De 1,25 milhão de pessoas em Hong Kong que receberam ao menos uma dose de CoronaVac entre fevereiro de 2021 e janeiro de 2022, 622 mil (49,6%) tinham ao menos 60 anos, sendo que 293 mil (47,9%) eram homens e 329 mil (52,9%) mulheres.

Entre os 622 mil idosos investigados, 126 mil (20,4%) receberam uma dose da CoronaVac, 384 mil (61,7%) tomaram duas doses e 111 mil (17,9%) receberam as três doses da vacina. Os dados são do Departamento de Saúde do governo de Hong Kong.

De acordo com os pesquisadores, a incidência de eventos adversos nos 21 dias seguintes à vacinação com a CoronaVac foi baixa. Entre todas as categorias de eventos adversos de interesse especial, foram registrados apenas 79,9 casos, todos leves, a cada grupo de 100 mil imunizados.

"Em comparação com o risco de mortalidade e complicações da Covid-19 em pessoas idosas, os benefícios da vacinação com a CoronaVac superam os riscos. Nossos resultados são consistentes com dados apontados em outros estudos com vacinas inativadas, que mostram sua segurança em outros grupos de risco, como imunossuprimidos e pessoas com hepatite B crônica", afirmam os autores.

Publicado em: 1/7/2022

Safety of an inactivated, whole-virion COVID-19 vaccine (CoronaVac) in people aged 60 years or older in Hong Kong: a modified self-controlled case series

Eric Yuk Fai Wan, Yuan Wang*, Celine Sze Ling Chui, Anna Hoi Ying Mok, Wanchun Xu, Vincent Ka Chun Yan, Francisco Tsz Tsun Lai, Xue Li, Carlos King Ho Wong, Esther Wai Yin Chan, Kui Kai Lau, Benjamin John Cowling, Ivan Fan Ngai Hung, Ian Chi Kei Wong*

Summary

Background Because evidence on the safety of COVID-19 vaccines in older adults is scarce, we aimed to evaluate the incidence and risk of adverse events after CoronaVac (Sinovac Biotech) vaccination in adults aged 60 years or older.

Methods In this modified self-controlled case series, we enrolled adults aged 60 years or older who had received at least one dose of CoronaVac in Hong Kong between Feb 23, 2021, and Jan 31, 2022. We extracted population-based, electronic health record data from the clinical management system of the Hospital Authority on adverse events of special interest (from Jan 1, 2005, to Feb 23, 2022) and patients' demographic information (from Jan 1, 2018, to Jan 31, 2022), previous diagnoses (from Jan 1, 2018, to Jan 31, 2022), medication history (from Jan 1, 2018, to Jan 31, 2022), and laboratory tests, including those for SARS-CoV-2 infection (from Jan 1, 2018, to Jan 31, 2022). Details of vaccination status were provided by the Department of Health of the Hong Kong Government and were linked to data from the Hospital Authority with identity card numbers or passport numbers. Our outcomes were the overall incidence of any adverse event of special interest and the incidence rates of 30 adverse events of special interest, as suggested by the WHO Global Advisory Committee on Vaccine Safety, in the inpatient setting within 21 days (2 days for anaphylaxis) of either the first, second, or third CoronaVac dose compared with a baseline period. Individuals who had a history of a particular event between Jan 1, 2005, and Feb 23, 2021, were excluded from the corresponding analysis. We evaluated the risk of an adverse event of special interest using conditional Poisson regression, adjusting for seasonal effects.

Findings Of 1 253 497 individuals who received at least one dose of CoronaVac during the study period, 622 317 (49·6%) were aged at least 60 years and were included in the analysis. Our analysis sample received 1 229 423 doses of CoronaVac and had a mean age of 70·40 years (SD 8·10). 293 086 (47·1%) of 622 317 participants were men and 329 231 (52·9%) were women. The incidence of individual adverse events of interest ranged from 0·00 per 100 000 people to 57·49 per 100 000 people (thromboembolism). The first and third doses of CoronaVac were not associated with a significant excess risk of an adverse event of special interest within 21 days (or 2 days for anaphylaxis) of vaccination. After the second dose, the only significantly increased risk was for anaphylaxis (adjusted incidence rate ratio 2·61, 95% CI 1·08–6·31; risk difference per 100 000 people 0·61, 95% CI 0·03–1·81).

Interpretation Because older age is associated with poor outcomes after SARS-CoV-2 infection, the benefits of CoronaVac vaccination in older adults outweigh the risks in regions where COVID-19 is prevalent. Ongoing monitoring of vaccine safety is warranted.

Funding The Food and Health Bureau of the Government, Hong Kong Special Administrative Region, China and AIR@InnoHK, administered by the Innovation and Technology Commission.

Lancet Healthy Longev 2022; 3: e491–500

See **Comment** page e455

*Co-first authors and contributed equally

For the Chinese translation of the abstract see **Online** for appendix 1

Centre for Safe Medication Practice and Research, Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine (E Y F Wan PhD, V K C Yan BPharm, F T T Lai PhD, X Li PhD, C K H Wong PhD, E W Y Chan PhD, Prof I C K Wong PhD), **Department of Family Medicine and Primary Care, School of Clinical Medicine, LiKa Shing Faculty of Medicine** (E Y F Wan, Y Wang MStat, A H Y Mok MClinPharm, W Xu MSc, C K H Wong), **School of Nursing, Li Ka Shing Faculty of Medicine** (C S L Chui PhD), **School of Public Health, Li Ka Shing Faculty of Medicine** (C S L Chui, Prof B J Cowling PhD), **Department of Medicine, School of Clinical Medicine, Li Ka Shing Faculty of Medicine** (X Li, K K Lau DPhil, Prof I F N Hung MD), **and State Key Laboratory of Brain and Cognitive Sciences** (K K Lau), **The University of Hong Kong, Hong Kong Special Administrative Region, China; Laboratory of Data Discovery for Health, Hong Kong Science and Technology Park, Hong Kong Special Administrative Region, China** (E Y F Wan, C S L Chui, F T T Lai, X Li, C K H Wong, E W Y Chan, Prof B J Cowling, Prof I C K Wong); **Expert Committee on Clinical Events Assessment Following Covid-19 Immunization, Department of Health, The Government of the Hong Kong Special Administrative Region,**

Introduction

Older age is a well recognised risk factor for complications after SARS-CoV-2 infection—individuals aged 60 years or older have a five-times increased risk of mortality after symptomatic SARS-CoV-2 infection compared with adults aged 30–59 years.[1] In view of this increased risk, older adults have been prioritised for COVID-19 vaccination in many countries and territories, including, but not limited to, the USA, the UK, and Hong Kong.[2] CoronaVac from Sinovac Biotech (Hong Kong; equivalent to Sinovac Life Sciences) has been available for emergency use in Hong Kong during the COVID-19 pandemic.

Two inactivated COVID-19 vaccines, namely CoronaVac and BBIBP-CorV (Sinopharm), have contributed to almost half of the COVID-19 vaccine doses administered around the globe.[3] Although accounting for around 75% of the COVID-19 vaccines administered in Brazil,[4] there have been few post-marketing clinical studies evaluating the safety of CoronaVac, especially in the older population. There have been infrequent reports of

Hong Kong Special Administrative Region, China (Prof I F N Hung, Prof I C K Wong); **Research Department of Practice and Policy, School of Pharmacy, University College London, London, UK** (Prof I C K Wong)

Correspondence to: Prof Ian Chi Kei Wong, Centre for Safe Medication Practice and Research, Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China
wongick@hku.hk

Research in context

Evidence before this study

We searched PubMed and Embase for articles published in English between database inception and March 23, 2022, using the search terms "adverse event", "older adults", "vaccines", and "CoronaVac". Most studies were case reports describing adverse events, such as ischaemic stroke and thrombotic events, after CoronaVac (Sinovac Biotech) vaccination. Evidence other than case reports is still sparse. Although randomised controlled trials of CoronaVac have not shown major adverse events after vaccination, our search did not identify any analytical studies on the risk of adverse events following CoronaVac vaccination in older adults.

Added value of this study

To our knowledge, this population-based study is the first to investigate the safety of CoronaVac in people aged 60 years or older. This self-controlled case series evaluates the risk of adverse events of special interest after the first, second, and third doses of CoronaVac vaccine. The self-controlled case series method was developed to investigate vaccine safety, and the advantage is that it minimises measured and unmeasured time-invariant confounding through within-individual comparisons. This study found that participants did not have a significantly higher risk of adverse events of special interest after CoronaVac vaccination compared with a baseline period, except for anaphylaxis after the second dose. However, the absolute risk increment for anaphylaxis was small.

Implications of all the available evidence

Given the extensive use of inactivated COVID-19 vaccines worldwide and the association of older age with poorer outcomes after SARS-CoV-2 infection, the potential risks of CoronaVac vaccination are outweighed by its benefits in places where COVID-19 is prevalent. More pharmacovigilance studies are warranted to confirm the safety of COVID-19 vaccines in older adults.

myocarditis in people aged 70 years after Ad.26.COV2.S (Janssen) and mRNA-1273 (Moderna) vaccination.[5,6] Some case reports also describe ischaemic stroke and thrombotic events after CoronaVac vaccination.[7,8] Nonetheless, evidence of post-vaccination adverse events in older adults beyond case reports is still scarce. Although randomised controlled trials of CoronaVac have not shown major adverse events after vaccination,[9,10] they only included a small proportion of older participants and were not able to evaluate rare events due to small numbers of events. People aged 60 years or older were excluded from the phase 3 trial of CoronaVac in Turkey[9] and represented the minority (37 [9%] of 434) of participants enrolled in the phase 3 trial of CoronaVac in Chile.[10] Despite the large number of CoronaVac doses being administered worldwide, vaccine-related adverse events might be under-reported in resource-limited areas,[11] rendering pharmacovigilance studies necessary.

Unlike myocarditis, which has been more frequently observed in young males who have received BNT162b2 (Pfizer–BioNTech) than in unvaccinated controls,[12] no significantly increased risk of adverse events has been found in the older vaccinated population versus the older unvaccinated population so far. One study[13] found that older adults (≥65 years) were more likely to be hospitalised after COVID-19 vaccination than were younger adults (18–64 years), but relatedness to vaccination is unknown because older adults generally have a higher risk of hospitalisation than do younger adults. Other studies have described a lower prevalence of local and systemic side-effects after BNT162b2 or ChAdOx1 nCov-19 (AstraZeneca) vaccination in participants older than 55 years (*vs* those aged ≤55 years)[14] or in participants aged 50 years or older (*vs* those aged 20–29 years).[15] Discrepancies and inconsistencies among previous studies reveal a need to evaluate vaccine safety in the older population. Whether the multiple comorbidities that are commonly seen in older people put them at higher risk of developing adverse reactions to COVID-19 vaccines is unknown. Older adults (aged ≥65 years) have reported reduced reactogenicity (local and systemic reactions) following mRNA-based COVID-19 vaccines compared with younger adults (aged <65 years),[16] hence raising the question of whether post-vaccination event rates might be different in older, compared with younger, adults. We aimed to examine the incidence and risk of adverse events of special interest after vaccination with CoronaVac in older adults.

Methods

Study design and participants

In this modified self-controlled case series, adults aged 60 years or older at the time of vaccination who had received CoronaVac (one dose or more) in Hong Kong between Feb 23, 2021, and Jan 31, 2022, were included. Adverse events of special interest were based on primary diagnoses upon hospitalisation. The self-controlled case series design relies on within-individual comparisons and is now an established study design for the evaluation of vaccine safety. This design has been applied in several studies of vaccine safety, including studies of COVID-19 vaccines.[17–21] The benefit of a self-controlled case series is that it treats individuals as their own control, thereby minimising measured or unmeasured time-invariant confounding.

Ethical approval for this study was granted by the Institutional Review Board of the University of Hong Kong and Hospital Authority Hong Kong West Cluster (UW21–149 and UW21–138) and the Department of Health Ethics Committee (LM21/2021). As anonymous data were extracted from an electronic health database, under Hong Kong regulations and

approval from the Hospital Authority and the Department of Health, consent from participants was not required. The study protocol is available online on the website of the COVID-19 Vaccines Adverse Events Response and Evaluation Programme.

For the **study protocol** see https://www.hkcare.hku.hk/

Data source

We extracted data from the clinical management system of the Hospital Authority, which stores electronic health records in Hong Kong, on adverse events of special interest (from Jan 1, 2005, to Feb 23, 2022) and patients' demographic information (from Jan 1, 2018, to Jan 31, 2022), previous diagnoses (from Jan 1, 2018, to Jan 31, 2022), medication history (from Jan 1, 2018, to Jan 31, 2022), and laboratory tests, including those for SARS-CoV-2 infection (from Jan 1, 2018, to Jan 31, 2022). Being a statutory administrative body in the Hong Kong Special Administrative Region, China, the Hospital Authority manages 43 public hospitals, 49 specialist outpatient clinics, and 73 primary care clinics in Hong Kong. Electronic health record data from all these Hospital Authority facilities, including emergency room visits, are captured by the clinical management system. The information recorded in the clinical management system of the Hospital Authority has been applied to several COVID-19 vaccine pharmacovigilance studies.[12,13,20,21]

For the **clinical management system of the Hospital Authority** see https://www.ha.org.hk/

Mortality data were extracted between Feb 23, 2021, and Jan 31, 2022, from the Deaths Registry under the Immigration Department of the Government of the Hong Kong Special Administrative Region, China, entries to which are mandatory for all residents in Hong Kong and are used by all Government departments in Hong Kong. The mass vaccination programme in Hong Kong was launched on Feb 23, 2021. Information regarding vaccination status and vaccine type from Feb 23, 2021, to Jan 31, 2022, was provided by the Department of Health of the Hong Kong Government.[22] Details of vaccination status were linked to pre-existing data in the clinical management system with a deidentified, unique Hong Kong identity card number or passport number for each participant.

For the **Deaths Registry** see https://www.immd.gov.hk/eng/services/birth-death-marriage-registration.html

Outcomes

Our outcomes were the overall incidence rate of any adverse event of special interest and the incidence rates of 30 adverse events of special interest, which were obtained from the list of events suggested by the WHO Global Advisory Committee on Vaccine Safety,[23] in the inpatient setting within 21 days (2 days for anaphylaxis) of either the first, second, or third CoronaVac vaccine dose compared with a baseline period. The adverse events of special interest were: autoimmune diseases (Guillain–Barré Syndrome, acute disseminated encephalomyelitis, narcolepsy, acute aseptic arthritis, type 1 diabetes, [idiopathic] thrombocytopenia, and subacute thyroiditis); cardiovascular diseases (microangiopathy, heart failure, stress cardiomyopathy, coronary artery disease, arrhythmia, and myocarditis); diseases of the circulatory system (thromboembolism, haemorrhagic disease, and single organ cutaneous vasculitis); diseases of the hepatorenal system (acute liver injury, acute kidney injury, and acute pancreatitis); diseases of peripheral nerves and the CNS (generalised convulsion, meningoencephalitis, transverse myelitis, and Bell's palsy); disease of the respiratory system (acute respiratory distress syndrome); diseases of the skin, mucous membranes, and joints (erythema multiforme and chilblain-like lesions); and other system diseases (anaphylaxis, anosmia, ageusia, Kawasaki disease, and rhabdomyolysis). These adverse events of special interest were based on a single principal inpatient diagnosis with procedure codes and codes in the International Classification of Diseases, Ninth Revision, Clinical Modification. The detailed definition of each adverse event of special interest is displayed in appendix 2 (p 4).

See Online for appendix 2

Statistical analysis

To achieve 80% power to detect an incidence rate ratio in the exposure period between 1·5 to 3·0 at the 0·05 significance level, the required sample size for our self-controlled case series analysis ranged from 32 to 278. More details on sample size calculation are provided in appendix 2 (pp 2–3).

Theoretically, three assumptions must be fulfilled when adopting the self-controlled case series model. To satisfy these assumptions, we used a modified self-controlled case series model and only considered the first incidence of a specific adverse event of special interest during the observation period, excluding subsequent episodes in the same participant. More detail on these assumptions can be found in appendix 2 (p 1).

When measuring the separate incidences of adverse events of special interest, individuals who had a history of a particular event between Jan 1, 2005, and Feb 23, 2021, were excluded from the corresponding analysis. For all adverse events of special interest except anaphylaxis, only the first incidence of an event in an individual within 21 days (inclusive of the day of vaccination) of the first, second, or third dose of CoronaVac (ie, 0–20 days post-vaccination) would be regarded as an incident adverse event of special interest. The duration of 21 days was chosen such that medium-term adverse events could be identified, while the risk of short-term outcomes would not be underestimated because of dilution caused by further extending the observation period.[24] Previous population-based pharmacovigilance studies that evaluated COVID-19 vaccine safety also adopted 21 days as the duration of the risk period.[24,25] Because classifying allergic reactions with a symptom onset of 2 days or more after vaccination would have been difficult, the risk period for anaphylaxis was defined as 2 days (ie, 0–1 day post-vaccination). This approach has also been applied by the Centers for Disease Control and Prevention in the USA when monitoring anaphylaxis events after BNT162b2

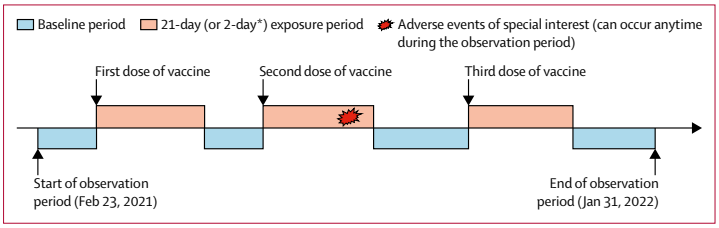


Figure 1: **Observation timeline of a hypothetical patient in the self-controlled case series**
*The exposure period was 2 days for anaphylaxis.

vaccination.[26] The incidences of adverse events of special interest are presented in three ways: incidence per 100 000 doses, incidence per 100 000 people, and incidence rate (cases per 100 000 person-days). 95% CIs for these measurements were calculated as the exact binomial CIs.[27] Futhermore, as the modified self-controlled case series design is not applicable for evaluating the risk of mortality after vaccination, we report all-cause mortality as a descriptive statistic.

Three exposure periods were considered in the self-controlled case series analysis: 0–20 days (0–1 day for anaphylaxis) after the first dose, 0–20 days (0–1 day for anaphylaxis) after the second dose, and 0–20 days (0–1 day for anaphylaxis) after the third dose of vaccine (figure 1). Other risk periods during the study period apart from the exposure periods were considered a baseline period (figure 1). The self-controlled case series analysis was done for a particular adverse event of special interest only when the overall number of events recorded for that event was at least five. To examine event-dependent exposure, the modified self-controlled case series model was applied by use of the R function *eventdepenexp* in the R package, SCCS. By comparing the incidence rates of adverse events of special interest in different risk periods with those in the baseline period, we calculated incidence rate ratios and corresponding 95% CIs using conditional Poisson regression, adjusting for seasonal effects in monthly categories. The model was not adjusted for any additional confounders because the self-controlled case series design does not require adjustment for any time-invariant confounders given that individuals serve as their own control. We checked the overdispersion assumption. Additionally, we calculated risk differences per 100 000 people using the difference in incidences between risk periods and the baseline period. Incidence in the risk periods was calculated by use of the observational data collected in this study, whereas incidence in the baseline period was calculated by dividing the incidence in the risk period by the adjusted incidence rate ratio.

Moreover, we did prespecified subgroup analyses of adjusted incidence rate ratios and risk differences, stratifying by age (<80 years *vs* ≥80 years) and Charlson Comorbidity Index (<3 *vs* ≥3), to confirm whether results were consistent among different age and comorbidity groups. Three prespecified sensitivity analyses were done to ensure robustness. In the first sensitivity analysis, individuals who were infected with SARS-CoV-2 before or during the study period were excluded, owing to the possible increased risk of post-vaccination adverse events of special interest after SARS-CoV-2 infection. In the second and final sensitivity analyses, the duration of exposure periods for all adverse events of special interest, except for anaphylaxis, was changed from 21 days to 14 days or 28 days, respectively, to investigate whether similar results could be reproduced.

All statistical tests were two-sided. R (version 4.0.3) was used to conduct all statistical analyses. At least two investigators (YW, WX, or VKCY) independently conducted each analysis for quality assurance.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Of 1 253 497 individuals who received CoronaVac in Hong Kong between Feb 23, 2021, and Jan 31, 2022, 622 317 (49·6%) were aged at least 60 years and were included in the analysis. Overall, 1 229 423 doses of CoronaVac were administered to these 622 317 people within the study period, among whom 126 736 (20·4%) had one dose, 384 056 (61·7%) had two doses, and 111 525 (17·9%) had three doses of the vaccine. The mean age of CoronaVac recipients was 70·40 years (SD 8·10), of whom 47·1% were men and 52·9% were women (table 1). Pre-existing comorbidities and medication use within the past 90 days among included individuals are shown in appendix 2 (p 5).

By and large, the incidences of adverse events of special interest and all-cause mortality within 21 days (or 2 days for anaphylaxis) of vaccination with CoronaVac were small (table 2). The incidence of individual adverse events of special interest ranged from 0·00 per 100 000 doses to 31·81 per 100 000 doses (thromboembolism), from 0·00 per 100 000 people to 57·49 per 100 000 people (thromboembolism), and from 0·00 per 100 000 person-days to 1·43 per 100 000 person-days (thromboembolism; table 2). Adverse events of special interest that were not observed within 21 days of vaccination were: type 1 diabetes; subacute thyroiditis; microangiopathy; stress cardiomyopathy; single organ cutaneous vasculitis; chilblain-like lesions; and Kawasaki disease. Only three adverse events of special interest—coronary artery disease, arrhythmia, and thromboembolism—had an incidence of more than 20 cases per 100 000 people (table 2).

The first and third doses of CoronaVac were not associated with a significant excess risk of an adverse event of special interest within 21 days (or 2 days for

	Overall (n=622 317)	CoronaVac recipients with 1 dose (n=126 736)	CoronaVac recipients with 2 doses (n=384 056)	CoronaVac recipients with 3 doses (n=111 525)
Demographics and comorbidities				
Age, years	70·40 (8·10)	73·81 (9·08)	69·69 (7·79)	68·95 (6·80)
Sex				
Female	329 231 (52·9%)	74 769 (59·0%)	205 705 (53·6%)	48 757 (43·7%)
Male	293 086 (47·1%)	51 967 (41·0%)	178 351 (46·4%)	62 768 (56·3%)
Charlson Comorbidity Index	3·00 (1·16)	3·43 (1·32)	2·92 (1·11)	2·76 (0·96)
History of adverse events of special interest				
Guillain–Barré Syndrome	921 (0·1%)	216 (0·2%)	568 (0·1%)	137 (0·1%)
Acute disseminated encephalomyelitis	18 (<0·1%)	6 (<0·1%)	11 (<0·1%)	1 (<0·1%)
Narcolepsy	16 067 (2·6%)	3134 (2·5%)	9362 (2·4%)	3571 (3·2%)
Acute aseptic arthritis	5145 (0·8%)	1517 (1·2%)	2953 (0·8%)	675 (0·6%)
Type 1 diabetes	179 (<0·1%)	59 (<0·1%)	102 (<0·1%)	18 (<0·1%)
Thrombocytopenia (idiopathic)	2099 (0·3%)	581 (0·5%)	1259 (0·3%)	259 (0·2%)
Subacute thyroiditis	71 (<0·1%)	17 (<0·1%)	41 (<0·1%)	13 (<0·1%)
Microangiopathy	16 (<0·1%)	4 (<0·1%)	11 (<0·1%)	1 (<0·1%)
Heart failure	10 391 (1·7%)	3810 (3·0%)	5635 (1·5%)	946 (0·8%)
Stress cardiomyopathy	0	0	0	0
Coronary artery disease	47 628 (7·7%)	12 971 (10·2%)	27 463 (7·2%)	7194 (6·5%)
Arrhythmia	30 377 (4·9%)	9397 (7·4%)	17 173 (4·5%)	3807 (3·4%)
Myocarditis	2128 (0·3%)	590 (0·5%)	1206 (0·3%)	332 (0·3%)
Thromboembolism	64 505 (10·4%)	18 835 (14·9%)	37 602 (9·8%)	8068 (7·2%)
Haemorrhagic disease	28 190 (4·5%)	8543 (6·7%)	16 532 (4·3%)	3115 (2·8%)
Single organ cutaneous vasculitis	1680 (0·3%)	453 (0·4%)	993 (0·3%)	234 (0·2%)
Acute liver injury	17 672 (2·8%)	3536 (2·8%)	11 183 (2·9%)	2953 (2·6%)
Acute kidney injury	16 256 (2·6%)	5099 (4·0%)	9203 (2·4%)	1954 (1·8%)
Acute pancreatitis	2646 (0·4%)	710 (0·6%)	1562 (0·4%)	374 (0·3%)
Generalised convulsion	3676 (0·6%)	1063 (0·8%)	2231 (0·6%)	382 (0·3%)
Meningoencephalitis	666 (0·1%)	169 (0·1%)	399 (0·1%)	98 (0·1%)
Transverse myelitis	24 (<0·1%)	8 (<0·1%)	15 (<0·1%)	1 (<0·1%)
Bell's palsy	4637 (0·7%)	1129 (0·9%)	2811 (0·7%)	697 (0·6%)
Acute respiratory distress syndrome	8243 (1·3%)	2391 (1·9%)	4792 (1·2%)	1060 (1·0%)
Erythema multiforme	139 (<0·1%)	30 (<0·1%)	80 (<0·1%)	29 (<0·1%)
Chilblain-like lesions	85 (<0·1%)	21 (<0·1%)	51 (<0·1%)	13 (<0·1%)
Anosmia or ageusia	688 (0·1%)	131 (0·1%)	418 (0·1%)	139 (0·1%)
Anaphylaxis	14 379 (2·3%)	3785 (3·0%)	8645 (2·3%)	1949 (1·7%)
Kawasaki disease	558 (0·1%)	127 (0·1%)	353 (0·1%)	78 (0·1%)
Rhabdomyolysis	902 (0·1%)	269 (0·2%)	539 (0·1%)	94 (0·1%)

Data are mean (SD) or n (%).

Table 1: **Baseline characteristics of CoronaVac recipients**

anaphylaxis) of vaccination (figure 2). However, we found a significantly increased risk of anaphylaxis (adjusted incidence rate ratio 2·61, 95% CI 1·08–6·31; risk difference per 100 000 people 0·61, 95% CI 0·03–1·81) within 2 days of the second vaccine dose compared with the baseline period (figure 2). No other significant increased risk of an adverse event of special interest was noted after the second dose (figure 2). There was no overdispersion for all adverse events of special interest as none of the outcome variables had larger variance values than mean values (appendix 2 p 7).

For our subgroup analyses, post-hoc, we grouped adverse events of special interest into disease categories (ie, autoimmune diseases, cardiovascular diseases, diseases of the circulatory system, diseases of the hepatorenal system, disease of the peripheral nerves and CNS, and disease of the respiratory system) due to rare incidences of individual events. Significant excess risk of any adverse event of special interest category was not observed after vaccination in those younger than 80 years or in those aged 80 years or older (appendix 2 p 8) or in those with a Charlson Comorbidity Index of less than 3

	n	Incidence per 100 000 doses (95% CI)	Incidence per 100 000 people (95% CI)	Incidence rate* (95% CI)
Any adverse event of special interest	668	79·97 (74·02–86·27)	145·45 (134·64–156·91)	3·59 (3·32–3·87)
Autoimmune disease	108	10·05 (8·25–12·14)	18·07 (14·82–21·81)	0·45 (0·37–0·55)
Guillain–Barré syndrome	1	0·09 (0·00–0·50)	0·16 (0·00–0·90)	0·00 (0·00–0·02)
Acute disseminated encephalomyelitis	1	0·09 (0·00–0·50)	0·16 (0·00–0·90)	0·00 (0·00–0·02)
Narcolepsy	80	7·35 (5·83–9·15)	13·20 (10·47–16·43)	0·33 (0·26–0·41)
Acute aseptic arthritis	26	2·35 (1·53–3·44)	4·21 (2·75–6·17)	0·11 (0·07–0·16)
Type 1 diabetes	0	0·00 (0·00–0·33)	0·00 (0·00–0·59)	0·00 (0·00–0·01)
Thrombocytopenia (idiopathic)	1	0·09 (0·00–0·50)	0·16 (0·00–0·90)	0·00 (0·00–0·02)
Subacute thyroiditis	0	0·00 (0·00–0·33)	0·00 (0·00–0·59)	0·00 (0·00–0·01)
Cardiovascular diseases	336	34·06 (30·52–37·91)	61·60 (55·19–68·55)	1·54 (1·38–1·71)
Microangiopathy	0	0·00 (0·00–0·33)	0·00 (0·00–0·59)	0·00 (0·00–0·01)
Heart failure	67	6·09 (4·72–7·73)	10·96 (8·49–13·91)	0·28 (0·21–0·35)
Stress cardiomyopathy	0	0·00 (0·00–0·33)	0·00 (0·00–0·59)	0·00 (0·00–0·01)
Coronary artery disease	161	15·56 (13·25–18·16)	28·05 (23·89–32·73)	0·70 (0·60–0·82)
Arrhythmia	166	15·58 (13·30–18·14)	28·08 (23·97–32·69)	0·70 (0·60–0·82)
Myocarditis	7	0·63 (0·25–1·29)	1·13 (0·45–2·33)	0·03 (0·01–0·06)
Circulatory system	352	35·31 (31·72–39·20)	63·85 (57·36–70·88)	1·59 (1·43–1·77)
Thromboembolism	320	31·81 (28·42–35·50)	57·49 (51·37–64·15)	1·43 (1·28–1·60)
Haemorrhagic disease	39	3·65 (2·59–4·98)	6·57 (4·67–8·98)	0·16 (0·12–0·22)
Single organ cutaneous vasculitis	0	0·00 (0·00–0·33)	0·00 (0·00–0·59)	0·00 (0·00–0·01)
Hepatorenal system	40	3·79 (2·71–5·16)	6·82 (4·87–9·29)	0·17 (0·12–0·23)
Acute liver injury	5	0·46 (0·15–1·07)	0·83 (0·27–1·93)	0·02 (0·01–0·05)
Acute kidney injury	19	1·74 (1·05–2·72)	3·14 (1·89–4·90)	0·08 (0·05–0·12)
Acute pancreatitis	18	1·62 (0·96–2·56)	2·91 (1·72–4·59)	0·07 (0·04–0·12)
Peripheral nerves and CNS	65	5·90 (4·55–7·52)	10·60 (8·18–13·51)	0·27 (0·21–0·34)
Generalised convulsion	17	1·53 (0·89–2·45)	2·75 (1·60–4·40)	0·07 (0·04–0·11)
Meningoencephalitis	2	0·18 (0·02–0·65)	0·32 (0·04–1·16)	0·01 (0·00–0·03)
Transverse myelitis	1	0·09 (0·00–0·50)	0·16 (0·00–0·90)	0·00 (0·00–0·02)
Bell's palsy	46	4·15 (3·04–5·53)	7·45 (5·45–9·94)	0·19 (0·14–0·25)
Respiratory system (acute respiratory distress syndrome)	56	5·08 (3·83–6·59)	9·12 (6·89–11·85)	0·23 (0·17–0·30)
Skin, mucous membranes, and joints	1	0·09 (0·00–0·50)	0·16 (0·00–0·90)	0·00 (0·00–0·02)
Erythema multiforme	1	0·09 (0·00–0·50)	0·16 (0·00–0·90)	0·00 (0·00–0·02)
Chilblain-like lesions	0	0·00 (0·00–0·33)	0·00 (0·00–0·59)	0·00 (0·00–0·01)
Others	8	0·72 (0·31–1·42)	1·29 (0·56–2·54)	0·03 (0·01–0·06)
Anosmia or ageusia	1	0·09 (0·00–0·50)	0·16 (0·00–0·90)	0·00 (0·00–0·02)
Kawasaki disease	0	0·00 (0·00–0·33)	0·00 (0·00–0·59)	0·00 (0·00–0·01)
Rhabdomyolysis	7	0·63 (0·25–1·29)	1·13 (0·45–2·32)	0·03 (0·01–0·06)
Anaphylaxis†	6	0·55 (0·20–1·20)	0·99 (0·36–2·15)	0·25 (0·09–0·54)
All-cause mortality	175	15·65 (13·42–18·15)	28·12 (24·11–32·61)	0·71 (0·61–0·82)

*Cases per 100 000 person-days. †The follow-up period for the incidence of anaphylaxis was 2 days (ie, 0–1 day post-vaccination) and anaphylaxis was not included in the calculation of the overall incidence of adverse events of special interest.

Table 2: **Incidence of adverse events of special interest and all-cause mortality among patients receiving CoronaVac vaccines**

or a Charlson Comorbidity Index of 3 or more (appendix 2 p 9). The results of our three sensitivity analyses were similar to our main findings (appendix 2 pp 10–12).

Discussion

Until now, safety data for CoronaVac have been insufficient. In this large-scale, self- controlled case series, adults aged 60 years or older did not have a significantly higher risk of adverse events of special interest after CoronaVac vaccination compared with a baseline period, except for anaphylaxis within 2 days of the second dose. The absolute risk increment for anaphylaxis after the second vaccine dose was only six cases per 1 million people. In comparison with the excess risk of mortality and complications from COVID-19 in people aged 60 years or older observed in previous studies,[1] the benefits of vaccination still exceed

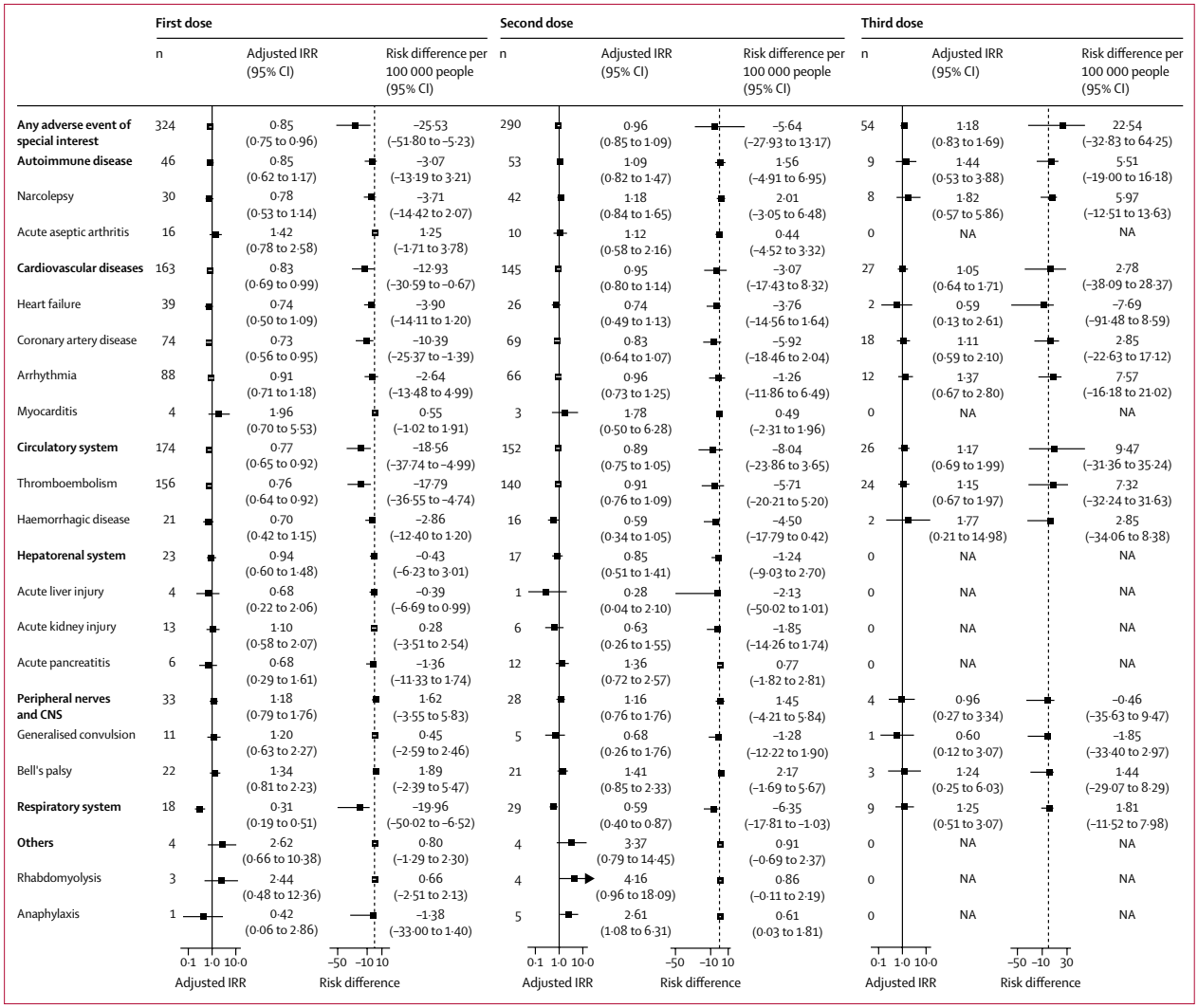


Figure 2: **Adjusted IRRs and risk differences of adverse events of special interest within 21 days of CoronaVac vaccination**
Adjusted IRRs were obtained from conditional Poisson regression adjusted for seasonal effects in our self-controlled case series analysis. The dashed line represents a risk difference of 0. IRR=incidence rate ratio. NA=not applicable.

its risks in places where COVID-19 is prevalent. Our results are consistent with the findings of previous studies of COVID-19 inactivated vaccines among a group of immunocompromised patients[4] and people with chronic hepatitis B virus infection.[28] Previous studies have revealed that multimorbidity does not translate to an additional risk of adverse events after COVID-19 vaccination[29] and that the composition of the gut microbiota might contribute to differences in post-vaccination adverse event rates.[30]

Anaphylaxis is an inherent risk associated with all vaccines and medicinal products. According to a previous study,[31] the estimated rate of anaphylaxis was 2·2 cases per 1 million doses of CoronaVac, which is slightly lower than our estimation (5·5 cases per 1 million doses [95% CI 2·0–12·0]). Allergic reactions can be directed towards the inactive excipients that stabilise the vaccine, such as polyethylene glycol and polysorbate, and, rarely, to the active component of the vaccine.[32] CoronaVac does not contain the aforementioned excipients,[33] yet theoretically carries a risk of anaphylaxis. Although it remains possible that we did not have sufficient statistical power to detect an increased risk of anaphylaxis after the first dose, the higher risk of anaphylaxis after the second

dose compared with the baseline period can potentially be attributed to a genuine anaphylactic reaction to the vaccine components, which only happens on re-exposure to the same allergen when it cross- links IgE on sensitised mast cells and triggers their degranulation.[34] Allergic reactions developing after the second dose, but not the first dose, of BNT162b2 have also been reported in the literature.[35] Because only 111 525 people in our study received a third dose of CoronaVac, it is very probable that our study did not have sufficient power to detect anaphylaxis after the third dose because the event rate was low. Despite anaphylaxis being potentially life-threatening, no deaths from allergic reactions after COVID-19 vaccination have been reported so far.[36]

The main result of our study—that there were no major adverse events after vaccination—is in accordance with findings from randomised controlled trials of CoronaVac done in populations mainly consisting of younger people (8·5% of participants were aged ≥60 years).[9,10] Some post-marketing observational studies[12,24,37–41] have raised specific concerns regarding the safety of CoronaVac and other COVID-19 vaccines using different platforms, such as mRNA and viral vectors. They have found associations with myocarditis following mRNA-based COVID-19 vaccines,[12,24,37,38] vascular events and thromboembolism following mRNA-based and viral vector-based COVID-19 vaccines,[39,40] and Bell's palsy following the CoronaVac COVID-19 vaccine.[41]

After vaccination with BNT162b2 or Ad.26.COV2.S vaccines, myocarditis in boys and men aged 12–24 years has been an issue of concern.[42,43] We did not find such an association in adults aged 60 years or older who received CoronaVac. As it is probably an immune-mediated reaction, post-vaccination myocarditis might be attributed to heightened immune responses in some clinically susceptible adolescents,[44] although the exact mechanism is not well understood. Our findings are in line with previous studies reporting a low incidence of myocarditis in older people after receiving mRNA vaccines.[45,46]

Although an increased risk of thromboembolism has been reported with BNT162b2 and the adenoviral vector vaccine ChAdOx1 nCoV-19,[39,40] we did not find an increased risk of thromboembolism with CoronaVac in our study; it is possible that we did not have sufficient statistical power to detect such rare events. With regards to the proposed mechanism of vaccine-associated thromboembolism, free DNA in the vaccine might trigger the production of antibodies against platelet factor 4, which in turn could activate platelets and promote immune thrombotic thrombocytopenia, resulting in bleeding or thrombosis.[47] As a whole-virion vaccine,[9] whether CoronaVac is associated with a lower risk of thromboembolism than other COVID-19 vaccines has been inadequately explored. A Thai study[48] revealed that CoronaVac recipients had a low prevalence of antibodies against platelet factor 4, but the relevance of this finding to vaccine-induced thrombotic thrombocytopenia is unknown. Presently, thromboembolic events are most common with ChAdOx1 nCoV-19,[39] with an estimated incidence of vaccine-induced immune thrombocytopenia and thrombosis of at least one case per 100 000 people aged 50 years or older.[49] As the approximate incidence of acute cerebrovascular disease in people with COVID-19 is 1·4% (95% CI 1·0–1·9),[50] the potential risk associated with vaccination is still substantially lower.

By contrast to a study[41] in Hong Kong that suggested that the risk of Bell's palsy in adults (aged ≥18 years) was increased after CoronaVac vaccination, we did not find an association between Bell's palsy and CoronaVac in recipients aged 60 years or older. This discrepancy could be ascribed to these events being rare in this older age group, resulting in inadequate power to detect such risk; previous studies report that the background incidence of Bell's palsy typically peaks at around 40–50 years of age.[51,52] More importantly, the risk of Bell's palsy is actually higher in those who are infected with SARS-CoV-2 than in COVID-19 vaccine recipients.[53] Indeed, current evidence regarding post-vaccination Bell's palsy remains largely inconsistent and limited in scope. Further studies with large sample sizes are needed to confirm our findings.

Our study has several strengths. First, in an area of sparse data, our study provides reassuring evidence regarding the safety of CoronaVac for adults aged 60 years or older. Second, we extracted data from the vaccine registry provided by the Department of Health of the Hong Kong Government, which covers the entire population of Hong Kong, and so our sample size was large and population-based. Third, the prevalence of COVID-19 in Hong Kong was low during the study period (ie, 14 197 COVID-19 cases confirmed as of Jan 31, 2022, among a population size of around 7·5 million),[54] and, therefore, the likelihood of SARS-CoV-2 infection interfering with post-vaccination reactions was minimal. Finally, our findings were robust to several sensitivity analyses.

Our study also has limitations. First, considering the relatively small number of events recorded, it is possible that this study did not have adequate statistical power to detect infrequent events. Second, we only enrolled patients who had ever attended clinics or hospitals under the Hospital Authority. Theoretically, people who had been vaccinated but had never used any public health-care service would not have been captured by our study. However, this number would have been reasonably small because more than 90% of inpatient care in Hong Kong is provided by the Hospital Authority.[55] Third, events might have been underdiagnosed or misclassified as they were defined by diagnostic codes in the database. Nevertheless, this limitation is probably minimal; the coding accuracy of the electronic health database of the Hospital Authority has been shown in previous studies in Hong Kong, showing that the positive predictive values for the diagnoses were high.[56–58] Fourth, no causal relationship can be established due to the observational nature of our study. Fifth, we cannot rule out the possibility that some

potentially vaccine-related events occurred outside the 21-day exposure period, and the list of adverse events of special interest that we adopted in this study is not exhaustive. Finally, we did not evaluate characteristics or predictors associated with an increased risk of adverse events of special interest after vaccination. Further studies are warranted to evaluate the long-term risks of COVID-19 vaccines and predictors for the risk of post-vaccination adverse events of special interest in the older population.

In summary, no increased risk of adverse events of special interest, except for anaphylaxis after the second dose, was detected in CoronaVac recipients aged 60 years or older. The absolute risk increment for anaphylaxis after the second vaccine dose was small. Because older age is associated with poor outcomes after SARS-CoV-2 infection, the benefits of vaccination in this population far outweigh the risks in places where COVID-19 is prevalent. More pharmacovigilance studies of COVID-19 vaccines among older people are warranted.

Contributors

EYFW and ICKW had the original idea for the study, contributed to the development of the study, extracted data from the source database, constructed the study design and the statistical model, reviewed the literature, and act as guarantors for the study. EYFW, YW, WX, and VKCY accessed and verified the data, and did the statistical analysis. EYFW, AHYM, and ICKW wrote the first draft of the manuscript. ICKW is the principal investigator and provided oversight for all aspects of this project. EYFW, YW, CSLC, AHYM, WX, VKCY, FTTL, EWYC, XL, CKHW, KKL, BJC, and IFNH provided critical input to the analyses, study design, and discussion. All authors contributed to the interpretation of the analysis, critically reviewed and revised the manuscript, and approved the final manuscript to be submitted. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

EYFW has received research grants from the Food and Health Bureau of the Hong Kong Government and the Hong Kong Research Grants Council, outside the submitted work. CSLC has received grants from the Food and Health Bureau of the Hong Kong Government, the Hong Kong Research Grants Council, the Hong Kong Innovation and Technology Commission, Pfizer, IQVIA, and Amgen, and personal fees from PrimeVigilance, outside the submitted work. EWYC reports honoraria from the Hospital Authority and grants from the Hong Kong Research Grants Council, the Research Fund Secretariat of the Food and Health Bureau, the National Natural Science Fund of China, the Wellcome Trust, Bayer, Bristol Myers Squibb, Pfizer, Janssen, Amgen, Takeda, and the Narcotics Division of the Security Bureau of Hong Kong Special Administrative Region, outside the submitted work. FTTL has been supported by the Research Grants Council Postdoctoral Fellowship under the Hong Kong Research Grants Council and has received research grants from the Food and Health Bureau of the Hong Kong Government, outside the submitted work. XL has received research grants from the Food and Health Bureau of the Hong Kong Government, the Research Grants Council Early Career Scheme, and the Research Grants Council Research Matching Grant Scheme; research and educational grants from Janssen and Pfizer; internal funding from the University of Hong Kong; and consultancy fees from Merck Sharp & Dohme, unrelated to this work. CKHW has received research grants from the Food and Health Bureau of the Hong Kong Government, the Hong Kong Research Grants Council, and the EuroQol Research Foundation, unrelated to this work. KKL received grants from the Research Fund Secretariat of the Food and Health Bureau, the Innovation and Technology Bureau, the Research Grants Council, Amgen, Boehringer Ingelheim, Eisai, and Pfizer, and consultation fees from Amgen, Boehringer Ingelheim, Daiichi Sankyo, and Sanofi, all outside the submitted work. BJC received consulting fees from AstraZeneca, Fosun Pharma, GSK, Moderna, Pfizer, Roche, and Sanofi Pasteur. IFNH received speaker fees from MSD. ICKW reports research funding from Amgen, Bristol Myers Squibb, Pfizer, Janssen, Bayer, GSK, Novartis, the Hong Kong Research Grants Council, the Hong Kong Health and Medical Research Fund, the National Institute for Health Research in England, the European Commission, and the National Health and Medical Research Council in Australia, outside the submitted work; has received speaker fees from Janssen and Medice in the previous 3 years; and is an independent non-executive director of Jacobson Medical in Hong Kong. All other authors declare no competing interests.

Data sharing

Data will not be made available to others because the data custodians have not given permission.

Acknowledgments

This study was supported by a research grant from the Food and Health Bureau of the Hong Kong Government (reference number COVID19F01). FTTL and ICKW's posts were partly funded by the Laboratory of Data Discovery for Health; hence, this work was partly supported by AIR@InnoHK, administered by the Innovation and Technology Commission. We acknowledge the contribution of colleagues from the Drug Office of the Department of Health of the Hong Kong Government and the Hospital Authority for providing vaccination and clinical data.

Editorial note: The Lancet Group takes a neutral position with respect to territorial claims in published maps and institutional affiliations.

References

1 Wu JT, Leung K, Bushman M, et al. Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China. *Nat Med* 2020; **26:** 506–10.

2 The Hong Kong Government. COVID-19 jab plan unveiled. Feb 18, 2021. https://www.news.gov.hk/eng/2021/02/20210218/20210218_155933_737.html (accessed March 1, 2022).

3 Mallapaty S. China's COVID vaccines have been crucial—now immunity is waning. *Nature* 2021; **598:** 398–99.

4 Medeiros-Ribeiro AC, Aikawa NE, Saad CGS, et al. Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial. *Nat Med* 2021; **27:** 1744–51.

5 Nassar M, Nso N, Gonzalez C, et al. COVID-19 vaccine-induced myocarditis: case report with literature review. *Diabetes Metab Syndr* 2021; **15:** 102205.

6 Kim HW, Jenista ER, Wendell DC, et al. Patients with acute myocarditis following mRNA COVID-19 vaccination. *JAMA Cardiol* 2021; **6:** 1196–201.

7 Hidayat R, Diafiri D, Zairinal RA, et al. Acute ischaemic stroke incidence after coronavirus vaccine in Indonesia: case series. *Curr Neurovasc Res* 2021; **18:** 360–63.

8 Hameed S, Khan AF, Khan S, Wasay M. First report of cerebral venous thrombosis following inactivated-virus covid vaccination (Sinopharm and Sinovac). *J Stroke Cerebrovasc Dis* 2022; **31:** 106298.

9 Tanriover MD, Doğanay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *Lancet* 2021; **398:** 213–22.

10 Bueno SM, Abarca K, González PA, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine in a subgroup of healthy adults in Chile. *Clin Infect Dis* 2021; published online Sept 19. https://doi.org/10.1093/cid/ciab823.

11 Murewanhema G, Dzinamarira T, Madziva R, Herrera H, Musuka G. SARS-CoV-2 vaccine-related adverse events in Zimbabwe: the need to strengthen pharmacovigilance in resource-limited settings. *Pharmacoepidemiol Drug Saf* 2021; **31:** 379–80.

12 Lai FTT, Li X, Peng K, et al. Carditis after COVID-19 vaccination with a messenger RNA vaccine and an inactivated virus vaccine: a case-control study. *Ann Intern Med* 2022; **175:** 362–70.

13 Xiong X, Yuan J, Li M, Jiang B, Lu ZK. Age and gender disparities in adverse events following COVID-19 vaccination: real-world evidence based on big data for risk management. *Front Med (Lausanne)* 2021; **8:** 700014.

14 Menni C, Klaser K, May A, et al. Vaccine side-effects and SARS-CoV-2 infection after vaccination in users of the COVID Symptom Study app in the UK: a prospective observational study. *Lancet Infect Dis* 2021; **21:** 939–49.

15 Kim S-H, Wi YM, Yun SY, et al. Adverse events in healthcare workers after the first dose of ChAdOx1 nCoV-19 or BNT162b2 mRNA COVID-19 vaccination: a single center experience. *J Korean Med Sci* 2021; **36:** e107.

16 Chapin-Bardales J, Gee J, Myers T. Reactogenicity following receipt of mRNA-based COVID-19 vaccines. *JAMA* 2021; **325:** 2201–02.

17 Whitaker HJ, Farrington CP, Spiessens B, Musonda P. Tutorial in biostatistics: the self-controlled case series method. *Stat Med* 2006; **25:** 1768–97.

18 Ghebremichael-Weldeselassie Y, Jabagi MJ, Botton J, et al. A modified self-controlled case series method for event-dependent exposures and high event-related mortality, with application to COVID-19 vaccine safety. *Stat Med* 2022; **41:** 1735–50.

19 Jabagi MJ, Botton J, Bertrand M, et al. Myocardial infarction, stroke, and pulmonary embolism after BNT162b2 MRNA COVID-19 vaccine in people aged 75 years or older. *JAMA* 2022; **327:** 80–82.

20 Wan EYF, Chui CSL, Wang Y, et al. Herpes zoster related hospitalization after inactivated (CoronaVac) and mRNA (BNT162b2) SARS-CoV-2 vaccination: a self-controlled case series and nested case-control study. *Lancet Reg Health West Pac* 2022; **21:** 100393.

21 Sing C-W, Tang CTL, Chui CSL, et al. COVID-19 vaccines and risks of hematological abnormalities: nested case-control and self-controlled case series study. *Am J Hematol* 2022; **97:** 470–80.

22 The Hong Kong Government. COVID-19 vaccination programme. 2021. https://www.covidvaccine.gov.hk/en/ (accessed March 1, 2022).

23 WHO. COVID-19 vaccines: safety surveillance manual. Establishing surveillance systems in countries using COVID-19 vaccines. 2020. https://www.who.int/vaccine_safety/committee/Module_AESI.pdf (accessed Sept 9, 2021).

24 Barda N, Dagan N, Ben-Shlomo Y, et al. Safety of the BNT162b2 mRNA Covid-19 vaccine in a nationwide setting. *N Engl J Med* 2021; **385:** 1078–90.

25 Klein NP, Lewis N, Goddard K, et al. Surveillance for adverse events after COVID-19 mRNA vaccination. *JAMA* 2021; **326:** 1390–99.

26 CDC COVID-19 Response Team, Food and Drug Administration. Allergic reactions including anaphylaxis after receipt of the first dose of Pfizer-BioNTech COVID-19 vaccine—United States, December 14–23, 2020. *MMWR Morb Mortal Wkly Rep* 2021; **70:** 46–51.

27 Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* 1934; **26:** 404–13.

28 Xiang T, Liang B, Wang H, et al. Safety and immunogenicity of a SARS-CoV-2 inactivated vaccine in patients with chronic hepatitis B virus infection. *Cell Mol Immunol* 2021; **18:** 2679–81.

29 Lai FTT, Huang L, Chui CSL, et al. Multimorbidity and adverse events of special interest associated with Covid-19 vaccines in Hong Kong. *Nat Commun* 2022; **13:** 411.

30 Ng SC, Peng Y, Zhang L, et al. Gut microbiota composition is associated with SARS-CoV-2 vaccine immunogenicity and adverse events. *Gut* 2022; **71:** 1106–16.

31 Laisuan W, Wongsa C, Chiewchalermsri C, et al. CoronaVac COVID-19 vaccine-induced anaphylaxis: clinical characteristics and revaccination outcomes. *J Asthma Allergy* 2021; **14:** 1209–15.

32 Kounis NG, Koniari I, de Gregorio C, et al. Allergic reactions to current available COVID-19 vaccinations: pathophysiology, causality, and therapeutic considerations. *Vaccines (Basel)* 2021; **9:** 221.

33 Turner PJ, Ansotegui IJ, Campbell DE, et al. COVID-19 vaccine-associated anaphylaxis: a statement of the World Allergy Organization Anaphylaxis Committee. *World Allergy Organ J* 2021; **14:** 100517.

34 Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012; **18:** 693–704.

35 Shavit R, Maoz-Segal R, Iancovici-Kidon M, et al. Prevalence of allergic reactions after Pfizer-BioNTech COVID-19 vaccination among adults with high allergy risk. *JAMA Netw Open* 2021; **4:** e2122255.

36 Krantz MS, Kwah JH, Stone CA Jr, et al. Safety evaluation of the second dose of messenger RNA COVID-19 vaccines in patients with immediate reactions to the first dose. *JAMA Intern Med* 2021; **181:** 1530–33.

37 Chua GT, Kwan MYW, Chui CSL, et al. Epidemiology of acute myocarditis/pericarditis in Hong Kong adolescents following Comirnaty vaccination. *Clin Infect Dis* 2021; published online Nov 28. https://doi.org/10.1093/cid/ciab989.

38 Li X, Lai FTT, Chua GT, et al. Myocarditis following COVID-19 BNT162b2 vaccination among adolescents in Hong Kong. *JAMA Pediatr* 2022; published online Feb 25. https://doi.org/10.1001/jamapediatrics.2022.0101.

39 Simpson CR, Shi T, Vasileiou E, et al. First-dose ChAdOx1 and BNT162b2 COVID-19 vaccines and thrombocytopenic, thromboembolic and hemorrhagic events in Scotland. *Nat Med* 2021; **27:** 1290–97.

40 Hippisley-Cox J, Patone M, Mei XW, et al. Risk of thrombocytopenia and thromboembolism after covid-19 vaccination and SARS-CoV-2 positive testing: self-controlled case series study. *BMJ* 2021; **374:** n1931.

41 Wan EYF, Chui CSL, Lai FTT, et al. Bell's palsy following vaccination with mRNA (BNT162b2) and inactivated (CoronaVac) SARS-CoV-2 vaccines: a case series and nested case-control study. *Lancet Infect Dis* 2022; **22:** 64–72.

42 Mevorach D, Anis E, Cedar N, et al. Myocarditis after BNT162b2 mRNA vaccine against Covid-19 in Israel. *N Engl J Med* 2021; **385:** 2140–49.

43 Diaz GA, Parsons GT, Gering SK, Meier AR, Hutchinson IV, Robicsek A. Myocarditis and pericarditis after vaccination for COVID-19. *JAMA* 2021; **326:** 1210–12.

44 Das BB, Moskowitz WB, Taylor MB, Palmer A. Myocarditis and pericarditis following mRNA COVID-19 vaccination: what do we know so far? *Children (Basel)* 2021; **8:** 607.

45 Witberg G, Barda N, Hoss S, et al. Myocarditis after Covid-19 vaccination in a large health care organization. *N Engl J Med* 2021; **385:** 2132–39.

46 Simone A, Herald J, Chen A, et al. Acute myocarditis following COVID-19 mRNA vaccination in adults aged 18 years or older. *JAMA Intern Med* 2021; **181:** 1668–70.

47 Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. *N Engl J Med* 2021; **384:** 2092–101.

48 Noikongdee P, Police P, Phojanasenee T, et al. Prevalence of anti-platelet factor 4/polyanionic antibodies after COVID-19 vaccination with ChAdOx1 nCoV-19 and CoronaVac in Thais. *Res Pract Thromb Haemost* 2021; **5:** e12600.

49 Pavord S, Scully M, Hunt BJ, et al. Clinical features of vaccine-induced immune thrombocytopenia and thrombosis. *N Engl J Med* 2021; **385:** 1680–89.

50 Nannoni S, de Groot R, Bell S, Markus HS. Stroke in COVID-19: a systematic review and meta-analysis. *Int J Stroke* 2021; **16:** 137–49.

51 Zhao H, Zhang X, Tang YD, Zhu J, Wang XH, Li ST. Bell's palsy: clinical analysis of 372 cases and review of related literature. *Eur Neurol* 2017; **77:** 168–72.

52 Tiemstra JD, Khatkhate N. Bell's palsy: diagnosis and management. *Am Fam Physician* 2007; **76:** 997–1002.

53 Cirillo N, Doan R. The association between COVID-19 vaccination and Bell's palsy. *Lancet Infect Dis* 2022; **22:** 5–6.

54 Centre for Health Protection. Latest situation of cases of COVID-19 (as of 31 January 2022). Hong Kong: Hong Kong Department of Health, 2022.

55 Leung GM, Wong IOL, Chan W-S, Choi S, Lo S-V. The ecology of health care in Hong Kong. *Soc Sci Med* 2005; **61:** 577–90.

56 Wong AYS, Root A, Douglas IJ, et al. Cardiovascular outcomes associated with use of clarithromycin: population based study. *BMJ* 2016; **352:** h6926.

57 Chan EW, Lau WCY, Leung WK, et al. Prevention of dabigatran-related gastrointestinal bleeding with gastroprotective agents: a population-based study. *Gastroenterology* 2015; **149:** 586–95.

58 Lau WCY, Chan EW, Cheung C-L, et al. Association between dabigatran *vs* warfarin and risk of osteoporotic fractures among patients with nonvalvular atrial fibrillation. *JAMA* 2017; **317:** 1151–58.

CoronaVac

O que a ciência comprova

5.2. CoronaVac reduziu casos graves e mortes por Covid-19 em idosos, diz estudo colombiano

Um estudo realizado na Colômbia com cerca de 700 mil participantes voltou a mostrar que a administração da CoronaVac em pessoas acima de 60 anos reduz de forma substancial as mortes e os casos graves de Covid-19. O trabalho foi publicado na The Lancet Regional Health – Americas e conduzido por pesquisadores da Universidade Nacional da Colômbia e das universidades colombianas de Sinú, de Cartagena e da Costa, além de outras instituições como a Universidade de São Paulo.

Entre março e agosto de 2021, os cientistas acompanharam 720 mil pessoas com idade média de 68 anos. Entre os voluntários, 76,7 mil haviam sido vacinados com CoronaVac, 56 mil tomaram outra vacina, e 539 mil ainda não haviam sido imunizados.

Nos indivíduos não vacinados, ocorreram 21,5 mil casos sintomáticos de Covid-19, 2.874 hospitalizações, 1.061 internações em Unidades de Terapia Intensiva (UTI) e 1.329 mortes. Já naqueles vacinados com a CoronaVac, o imunizante reduziu em mais da metade o risco de internação e morte por Covid-19.

Os autores do artigo chamam atenção para outro estudo conduzido na Colômbia com cerca de 3 milhões de pessoas, que mostrou uma efetividade ainda maior da vacina para evitar óbitos em idosos (72,1%). De acordo com a pesquisa publicada na The Lancet Healthy Longevity, menos de 1% dos idosos que tomaram CoronaVac morreram ou precisaram ser hospitalizados por consequências da Covid-19.

Segundo os pesquisadores, a diferença dos resultados pode ser explicada pelo menor poder estatístico da atual amostra (720 mil contra 3 milhões) e também pela realidade socioeconômica dos voluntários. Enquanto o trabalho anterior foi feito com toda a população colombiana, a atual pesquisa incluiu apenas participantes do norte do país com baixa condição socioeconômica – um público que corre mais risco de ser infectado pelo SARS-CoV-2 e tem menos acesso aos serviços de saúde.

A relação entre a Covid-19 e a vulnerabilidade social já foi demonstrada em diferentes países. Mais recentemente, um estudo da Universidade de São Paulo feito na Amazônia mostrou que crianças que passaram fome tiveram 76% mais chance de ter Covid-19 sintomática.

Publicado em: 1/7/2022

Effectiveness of CoronaVac and BNT162b2 COVID-19 mass vaccination in Colombia: A population-based cohort study

Angel Paternina-Caicedo,[a]* Mark Jit,[b] Nelson Alvis-Guzmán,[c,d] Juan Carlos Fernández,[e] José Hernández,[e] Justo Jesus Paz-Wilches,[e] José Rojas-Suarez,[c,f] Carmelo Dueñas-Castell,[c] Nelson J. Alvis-Zakzuk,[d,g] Adrian D. Smith,[h] and Fernando De La Hoz-Restrepo [i]

[a]Universidad del Sinú, Cartagena, Colombia
[b]London School of Hygiene & Tropical Medicine, London, United Kingdom
[c]Universidad de Cartagena, Cartagena, Colombia
[d]Universidad de la Costa – CUC, Barranquilla, Colombia
[e]Mutual Ser, Cartagena, Colombia
[f]Corporación Universitaria Rafael Núñez, Cartagena, Colombia
[g]Universidade de São Paulo, São Paulo, Brazil
[h]University of Oxford, Oxford, United Kingdom
[i]Universidad Nacional de Colombia, Bogotá, Colombia

The Lancet Regional Health - Americas 2022;12: 100296
Published online 1 July 2022
https://doi.org/10.1016/j.lana.2022.100296

Summary

Background In February 2021, Colombia began mass vaccination against COVID-19 using mainly BNT162b2 and CoronaVac vaccines. We aimed to estimate vaccine effectiveness (VE) to prevent COVID-19 symptomatic cases, hospitalization, critical care admission, and deaths in a cohort of 796,072 insured subjects older than 40 years in northern Colombia, a setting with a high SARS-CoV-2 transmission.

Methods We identified individuals vaccinated between March 1st of 2021 and August 15th of 2021. We included symptomatic cases, hospitalizations, critical care admissions, and deaths in patients with confirmed COVID-19 as main outcomes. We calculated VE for each outcome from the hazard ratio in Cox proportionally hazards regressions (adjusted by age, sex, place of residence, diabetes, human immunodeficiency virus, cancer, hypertension, tuberculosis, neurological diseases, and chronic renal disease), with 95% confidence intervals (CI).

Findings A total of 719,735 insured participants of 40 and more years were followed. We found 21,545 laboratory-confirmed symptomatic COVID-19 among unvaccinated population, along with 2874 hospitalizations, 1061 critical care admissions, and 1329 deaths, for a rate of 207.2 per million person-days, 27.1 per million person-days, 10.0 per million person-days, and 12.5 per million person-days, respectively. We found CoronaVac was not effective for any outcome in subjects above 80 years old; but for people 40-79 years of age, we found two doses of CoronaVac reduced hospitalization (33.1%; 95% CI, 14.5–47.7), critical care admission (47.2%; 95% CI, 18.5–65.8), and death (55.7%; 95% CI, 32.5–70.0). We found BNT162b2 was effective for all outcomes in the entire population of subjects above 40 years of age, significantly declining for subjects ≥80 years.

Interpretation Two doses of either CoronaVac in population between 40 and 79 years of age, or BNT162b2 among vaccinated above 40 years old significantly reduced deaths of confirmed COVID-19 in a cohort of individuals from Colombia. Vaccine effectiveness for CoronaVac and BNT162b2 declined with increasing age.

Funding UK National Institute for Health Research, the European Union's Horizon 2020 research and innovation programme, and the Bill & Melinda Gates Foundation.

Keywords: Vaccine effectiveness; Covid-19; Mortality; Vaccines

*Corresponding author at: Tv. 54 #41-117, Universidad del Sinú, Cartagena, Colombia.
E-mail address: apaterninac@unisinucartagena.edu.co (A. Paternina-Caicedo).

Introduction

The SARS-CoV-2 virus first appeared in Wuhan, China in late-2019, causing a cluster of cases of COVID-19

Articles

Research in context

Evidence before this study

We systematically reviewed the evidence using a MEDLINE search through PubMed, with the combination of keywords "trial" AND ("coronavac" OR "sinovac" OR "pfizer" OR "bnt162b2") AND ("vaccine" OR "vaccination") AND ("covid" OR "coronavirus" OR "SARS-CoV-2"). In addition, we made a Google search, and used the Johns Hopkins' International Vaccine Access Center systematic review of all studies reporting the effectiveness of COVID-19 vaccines. We included Phase 3 clinical trials in humans, published up to July 18th of 2021. We found six Phase 3 trials, one for CoronaVac (efficacy for symptomatic case of 83·5%; 95% confidence interval [CI], 65·4–92·1), and two for BNT162b2 (efficacies for symptomatic cases at all ages of 95·0 [95% CI, 90·3 to 97·6]; and 100·0% [95% CI, 75·3 to 100·0] in adolescents). For CoronaVac, there were two studies reporting effectiveness: one cohort study in Chile and a test-negative matched case-control design in Brazil. Both these latter studies show significant effectiveness to prevent death with CoronaVac vaccination.

Added value of this study

We report a significant reduction of death, critical care admission, and hospitalization for COVID-19, 14 days after the second dose of CoronaVac or BNT162b2 in a population 40–79 years old in Colombia, where at the time of this study, the Mu variant of interest was predominant. However, effectiveness against cases was negative for CoronaVac.

Implications of all the available evidence

BNT162b2 showed greater reductions than CoronaVac in death, critical care admission, and hospitalization for COVID-19 disease. Strengthening and increasing the speed of the immunization rollout with these vaccines may save lives in countries worldwide.

that quickly disseminated worldwide to become the first large pandemic of the 21st century.[1] As of May 17th, 2022, COVID-19 has killed at least 6·3 million people worldwide.[1]

Several efficacious and safe vaccines have been shown to prevent adverse outcomes due to COVID-19. The BNT162b2 vaccine has a reported efficacy of 95% (95% confidence interval [CI], 90.3–97.6) to prevent symptomatic cases,[2] while the mRNA-1273 vaccine has reported 94% (95% CI, 89·3–96·8) efficacy for this outcome.[3]

CoronaVac has seen less peer-reviewed scrutiny of its efficacy, effectiveness, and safety, yet has been widely deployed in 72 countries by January 28th of 2022. Studies for CoronaVac have shown large heterogeneity in vaccine effectiveness. A recent clinical trial in Turkey showed CoronaVac had an efficacy to prevent symptomatic cases of 83·5% (95% CI 65·4–92·1), whilst a trial in Brazil showed a 51% vaccine efficacy against cases (95% CI, 36–62). A matched negative-control case-control study showed CoronaVac had an effectiveness of 47% to prevent COVID-19 cases, and 61% to prevent COVID-19 deaths in Brazil.[4] Data from Chile show CoronaVac has an effectiveness of 66% to prevent cases.[5] Based on this data, the Strategic Advisory Group of Experts on Immunization of the World Health Organization approved CoronaVac for emergency use in countries worldwide.[6]

Colombia started vaccinating healthcare workers in early February 2021, mainly using the BNT162b2 vaccine. The CoronaVac vaccine was then used to vaccinate people older than 80 years, and later expanded to those older than 60 years.[7] After prioritization by occupations with higher risk of exposure, older age, and comorbidities, the vaccine was freely available in the country to the entire population without out-of-pocket expenses. As of 15th of August of 2021, overall vaccination coverage in Colombia with either vaccine was 40·8% for first doses and 27·1% for fully vaccinated individuals.

Given the need for real-world evidence on CoronaVac effectiveness, we consolidated data sources from one of the largest healthcare insurers in Colombia to provide evidence of the effectiveness of the vaccine in this setting. The present analysis provides a unique opportunity to assess vaccine effectiveness of CoronaVac alongside BNT162b2 COVID-19 vaccine in a setting where the Mu variant predominated in 2021. We aim here to assess the effectiveness of CoronaVac and BNT162b2 to prevent symptomatic cases, hospitalizations, critical care admissions, and deaths in patients with COVID-19 in Colombia.

Methods

Study design

We designed a retrospective cohort study to evaluate the effectiveness of the CoronaVac and BNT162b2 vaccination in Colombia between March 1st and August 15th of 2021. We only included insured subjects older than 40 years (Table 1) and excluded subjects with confirmed previous SARS-CoV-2 infection.

This study was approved by the ethics committee of Universidad del Sinú, Cartagena, Colombia.

Participants and data sources

Our study population is the entire population enrolled in Mutual Ser, a health insurer of around 2·15 million people from Colombia. The insured are mostly low- and middle-income populations whose healthcare claims are subsidized by the government. The healthcare system in Colombia, includes healthcare provision for poor population (subsidized regime), for people who work

Parameters	Entire cohort *n = 719735 (%)*	Unvaccinated *n = 539010 (%)*	Two-dose CoronaVac *n = 76729 (%)*	Two-dose BNT162b2 *n = 56140 (%)*
Age (yrs), *n* (%)				
40–49	213468 (29.7)	197166 (36.6)	1503 (2.0)	5086 (9.1)
50–59	215482 (29.9)	166090 (30.8)	13422 (17.5)	21073 (37.5)
60–69	125601 (17.5)	73470 (13.6)	19011 (24.8)	20355 (36.3)
70–79	79877 (11.1)	41849 (7.8)	22788 (29.7)	8373 (14.9)
80+	85307 (11.9)	60435 (11.2)	20005 (26.1)	1253 (2.2)
Sex, *n* (%)				
Female	369599 (51.4)	272186 (50.5)	40004 (52.1)	31024 (55.3)
Male	350136 (48.6)	266824 (49.5)	36725 (47.9)	25116 (44.7)
Type of municipality, *n* (%)				
Other cities	475075 (66.0)	346473 (64.3)	58575 (76.3)	38188 (68.0)
Capital city	244660 (34.0)	192537 (35.7)	18154 (23.7)	17952 (32.0)
Comorbidity, *n* (%)				
Diabetes	42556 (5.9)	25265 (4.7)	7795 (10.2)	5591 (10.0)
HIV	2404 (0.3)	1851 (0.3)	136 (0.2)	232 (0.4)
Hypertension	95893 (13.3)	53722 (10.0)	21182 (27.6)	12106 (21.6)
Cancer	9308 (1.3)	5870 (1.1)	1679 (2.2)	968 (1.7)
Tuberculosis	110 (<0.1)	89 (<0.1)	6 (<0.1)	8 (<0.1)

***Table 1*: Baseline characteristics of people vaccinated with two doses of CoronaVac and BNT162b2, and unvaccinated matched controls.**

(contributive regime), for military, teachers, and other populations (special regime), and for those who are willing to pay for private attention (out-of-pocket or private expenditure). Mutual Ser provides comprehensive health-related services under the subsidize regime for 25% in population of the Colombian departments of Atlántico, Bolívar, Cordoba, Magdalena, and Sucre. In 779 samples analyzed by the Colombian National Institute of Health, the Mu variant was predominant during this time-period (Figure 1). The data on variant frequency are publicly available.[8]

We identified comorbidities in insured patients (diabetes, human immunodeficiency virus (HIV), cancer, hypertension, tuberculosis, neurological diseases, and chronic renal diseases). To classify the diseases, we searched for diagnostic codes using the 10th version of the International Classification of Diseases (Supplementary Table S1), in the diagnosis of healthcare attentions in the insurer. We also used special health programs within the insurer to detect people with HIV, diabetes, hypertension, or cancer.

All persons with respiratory symptoms presenting to care were tested for COVID-19, as well as their contacts. The contact tracing algorithm searched for home family members and tested them, with or without symptoms.

Vaccination status and outcome assessment

The second dose of CoronaVac is given 28 days after the first dose, while the second dose of BNT162b2 is scheduled 21 days after the first dose.

We extracted records of vaccination receipt from a national dataset of vaccinated individuals in Colombia covering the period March 1st to August 15th of 202.[9,10] Vaccination records are collected by healthcare personnel administering the vaccine in health centers across the country, which enter them into a national official dataset of vaccinations. The records of the national dataset of vaccinations may be delayed therefore we collected the records of vaccinations two months after the end of the follow up period.

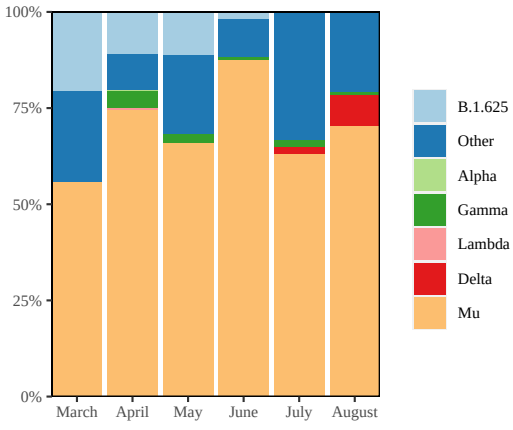


Figure 1. Monthly distribution of identified variants of SARS-CoV-2 in Colombia during the study period.

Note: This is the monthly distribution of variants in the region of the study period in Colombia, with 779 samples analyzed by the Colombian National Institute of Health.[12] These data are openly accessible. Samples without identified variants were excluded.

Articles

We defined four outcomes among persons with laboratory confirmed COVID-19: symptomatic illness, hospitalization, critical care admission and death, and extracted the dates of symptoms start from epidemiological records collected by Mutual Ser. Confirmatory laboratory testing comprised polymerase chain reaction (PCR), antigen, or IgM positive test. Incident cases were identified from both active and passive surveillance for COVID-19 symptoms (i.e., patients with cough, fever, difficult breathing, sore throat, or fatigue during the last past five days). Active surveillance was used by the insurer to trace and follow up contacts of laboratory-confirmed, symptomatic COVID-19 cases until free of symptoms. Hospitalizations and critical care admissions were collected from a dataset of all COVID-19 related healthcare attendances through a database collected by the insurer.

Person-times of follow-up

The follow-up started on March 1st of 2021 for all patients. All patients were unvaccinated at this date, and vaccinated patients before this date were excluded. Once a subject was vaccinated (14 days after the first dose), this person changed status to vaccinated with the first dose of either BNT162b2 or CoronaVac. Then, if the person was vaccinated with two doses (14 days after the second dose), the person changed status to fully vaccinated. We only assessed the effectiveness of the second dose. This means a person can contribute to person-times as unvaccinated or fully vaccinated, depending on the dates of the second dose of each vaccine.

Person-times ended on the start of symptoms, when the patient had a positive symptomatic SARS-CoV-2 test, and when the patient was hospitalized, admitted to critical care, or died. These subjects were censured if these outcomes did not present at the end of follow-up on August 15th, 2021.

Statistical analysis

We described the time to event for each outcome for cohorts using Kaplan–Meier curves. We fitted a Cox regression model with a vaccination status modeled as time-varying, to estimate the hazard ratios (HR) with 95% confidence intervals (CI). We tested the proportional hazards assumption using log-log plots. Vaccine effectiveness was calculated as one minus the HR. The analyses were reported crude and adjusted (by age, comorbidity, sex, and municipality). We also performed analyses to assess the potential differences of effectiveness depending on calendar time. For this assessment, we split the cohort into two analyses, one from March 1st until May 9th of 2021, and another cohort from May 10th to August 15th of 2021. These dates were chosen by diving the total study time of the main analysis into two halves. Both these cohorts had person-times depending on their start and end dates. The vaccine effectiveness in the split cohort was estimated similarly to the main analysis.

We stratified analyses by age (40–79, 80 years old or more), sex, and by presence or absence of comorbidities (diabetes, human immunodeficiency virus, cancer, hypertension, tuberculosis, neurological diseases, or chronic renal diseases), adding an interaction between vaccine doses and the strata (older age, sex, and comorbidity) to estimate the *p*-value of the difference between vaccine effectiveness in each stratum.

A *p*-value <0.05 was considered statistically significant for all analyses. All analyses were made in R (version 4.1.1), using the package 'survival' (version 3.-211).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Population

A total of 719,735 insured participants of 40 and more years were part of the cohort followed up from 1st of March of 2021 to August 15th of 2021, with a median age of 56 years old (IQR, 48––68), and 48·6% males (Table 1). The vaccine coverage for the first dose of all vaccines in the cohort was 25·1% at the end of follow-up.

The frequency of comorbidities is listed in Table 1, where hypertension was the most frequent comorbid disease (n = 95,893; 13·3%), followed by diabetes (n = 42,556; 5·9%).

We found 21,545 laboratory-confirmed symptomatic COVID-19 among unvaccinated population, along with 2874 hospitalizations, 1061 critical care admissions, and 1329 deaths, for a rate of 207·2 per million person-days, 27·1 per million person-days, 10·0 per million person-days, and 12·5 per million person-days, respectively.

Effectiveness of CoronaVac

The rate of disease among the two-dose cohort of CoronaVac was 205·1 per million person-days for symptomatic cases, 28·4 per million person-days for hospitalizations, 9·3 per million person-days for critical care admissions, and 13·6 per million person-days for deaths.

The effectiveness of CoronaVac was significantly different according to age and the presence of comorbidities. We found CoronaVac was not effective for any outcome in the totality of subjects above 40 years old; but for people younger than 80 years of age, we found two doses of CoronaVac reduced hospitalization (33·1%; 95% CI, 14·5 –47·7), critical care admission (47·2%; 95% CI, 18·5 –65·8), and death (55·7%; 95% CI, 32·5–70·0). See Tables 2 and 3. In our analyses, subjects with

Outcome and period	Laboratory-confirmed symptomatic Covid-19 Effectiveness (95% CI)*	Non-confirmed symptomatic Covid-19 illness Effectiveness (95% CI)*
CoronaVac vaccination		
Symptomatic case	-44.0 (-54.1 to -34.6)	-45.1 (-53.6 to -37.1)
Hospitalization	3.3 (-15.1 to 18.7)	-3.0 (-18.0 to 10.1)
Critical care admission	18.0 (-10.6 to 39.2)	13.6 (-13.2 to 34.0)
Death	21.4 (-0.7 to 38.6)	20.6 (-0.5 to 37.3)
BNT162b2 vaccination		
Symptomatic case	29.6 (21.1 to 37.2)	13.0 (5.3 to 20.0)
Hospitalization	54.2 (34.6 to 67.9)	45.5 (29.4 to 58.0)
Critical care admission	82.1 (56.5 to 92.6)	82.2 (60.1 to 92.1)
Death	93.5 (73.9 to 98.4)	94.1 (76.4 to 98.5)

***Table 2*: Effectiveness of two-dose vaccination with CoronaVac and BNT162b2 against Covid-19 cases, hospitalizations, critical care admissions, and deaths in Colombia.**
Note: * Vaccine effectiveness (95% confidence intervals).

comorbidities had significantly more CoronaVac effectiveness than subjects without these comorbid diseases (COVID-19 deaths were reduced among vaccinated with comorbidities by 44·6%; 95% CI, 20·6 to 61·3). Males had less CoronaVac effectiveness for COVID-19 symptomatic case and hospitalization (Table 3), without significant changes in the reduction of critical care admission or death.

In the analysis splitting the cohort to assess the effects of calendar time, CoronaVac reduced confirmed COVID-19 deaths in the first half of study-time by 17·2% (95% CI, -90·2 to 63·9) and 20·1% (95% CI, -8·8 to 41·30) in the second half. All the remaining outcomes were also non-significant in both cohorts split by half the calendar time of total study time.

Effectiveness of BNT162b2

The rate of disease among the two-dose cohort of CoronaVac was 108·4 per million person-days for

Scenarios	Symptomatic case Effectiveness (95% CI)*	Hospitalization Effectiveness (95% CI)*	Critical care admission Effectiveness (95% CI)*	Death Effectiveness (95% CI)*
		CoronaVac		
Age (yrs)				
40–79	-22.1 (-32.8 to -12.3)	33.1 (14.5 to 47.7)	47.2 (18.5 to 65.8)	55.7 (32.5 to 71.0)
80+	-149.2 (-185.2 to -117.8) (<0.001)	-76.5 (-133.3 to -33.5) (<0.001)	-50.8 (-141.5 to 5.9) (<0.001)	-19.3 (-66.2 to 14.3) (<0.001)
Sex				
Female	-36.6 (-49.7 to -24.7)	20.8 (-3.8 to 39.5)	32.9 (-9.3 to 58.8)	35.2 (4.2 to 56.2)
Male	-51.4 (-67.3 to -37.0) (<0.001)	-12.5 (-41.4 to 10.5) (<0.001)	6.4 (-37.0 to 36.1) (0.080)	9.0 (-25.5 to 34.1) (0.072)
Comorbidities				
Without comorbidities	-74.6 (-92.2 to -58.7)	-23.6 (-59.7 to 4.3)	-13.4 (-69.4 to 24.1)	-17.1 (-64.4 to 16.6)
With comorbidities	-21.3 (-33.4 to -10.2) (<0.001)	19.3 (-2.2 to 36.3) (0.005)	36.7 (1.0 to 59.5) (0.002)	44.6 (20.6 to 61.3) (<0.001)
		BNT162b2		
Age (yrs)				
40–79	32.2 (23.9 to 39.7)	59.7 (41.4 to 72.3)	85.9 (62.0 to 94.7)	96.7 (76.6 to 99.5)
80+	-48.5 (-178.7 to 20.8) (0.032)	-69.0 (-434.3 to 46.5) (0.019)	-30.0 (-850.6 to 82.2) (0.031)	34.0 (-374.8 to 90.8) (0.024)
Sex				
Female	31.2 (20.2 to 40.7)	66.0 (40.8 to 80.5)	87.1 (47.7 to 96.8)	93.9 (56.2 to 99.1)
Male	28.2 (14.0 to 40.0) (0.189)	40.6 (5.5 to 62.7) (0.183)	76.5 (26.0 to 92.5) (0.667)	93.2 (51.1 to 99.0) (0.991)
Comorbidities				
Without comorbidities	30.3 (17.5 to 41.2)	72.5 (44.6 to 86.4)	71.6 (22.6 to 89.6)	N.E.
With comorbidities	28.4 (16.1 to 38.8) (0.909)	40.7 (9.8 to 61.0) (0.128)	78.3 (47.2 to 91.1) (0.368)	87.2 (48.4 to 96.8) (0.980)

***Table 3*: Effectiveness of CoronaVac and BNT162b2 to prevent laboratory-confirmed symptomatic cases, hospitalizations, critical care, and deaths in Colombia, 14 days after the second dose, according to several scenarios.**
Note: * Vaccine effectiveness (95% confidence intervals) (*p*-value of the difference between the strata).
N.E.: Not estimable.

symptomatic cases, 10·8 per million person-days for hospitalizations, 1·7 per million person-days for critical care admissions, and 0·7 per million person-days for deaths.

We found BNT162b2 was effective for all outcomes in the entire population of subjects above 40 years of age (Table 2). We found that BNT162b2 effectiveness was different according to age, with older population having less reduction of all outcomes, while all other data stratification (comorbidities or not, and sex) did show significant differences and vaccination effectiveness when using two doses of BNT162b2 (Table 3).

The outcome with the highest BNT162b2 vaccine effectiveness with two doses in the entire population was death (93.5%; 95% CI, 73·9–98·4) (Table 2).

In the analysis stratifying the cohort by calendar time, the effectiveness of BNT162b2 was inestimable in the Cox proportionally hazards regression for COVID-19 deaths and critical care admissions. For COVID-19 confirmed symptomatic case, the effectiveness was 11·1% (95% CI, -532·2 to 87·5) in the first half and 43·3% (95% CI, 27·3 to 55·8) in the second half.

Discussion

We found a COVID-19 mortality risk reduction of 93·5% (95% CI, 73·9 to 98·4) for those with two doses of BNT162b and 55·7% (95% CI, 32·5 to 71·0) among those between 40 and 79 years of age vaccinated with two doses of CoronaVac. The effectiveness of CoronaVac and BNT162b2 estimated in the present study must be put into the context of the population studied. Our cohort of CoronaVac vaccinees is older than previous evaluations, with a median age of 68 years. This may bias towards lower effectiveness, especially for CoronaVac, which is reported to have lower effectiveness in older ages.[11] Our evaluation of the effectiveness of BNT162b2 is in line with previous studies showing a strong effectiveness for this vaccine.

Our results would also need to be put into the context of high pre-existing SARS-CoV-2 immunity through previous natural infection in the region of the study. In serosurveys prior to the start of the present analysis (in late-November of 2020), a high prevalence of antibodies against SARS-CoV-2 was found in two of the capital cities in this region, with a 59% seroprevalence in Monteria (capital of Cordoba) and 53% in Barranquilla (capital of Atlántico).[12] The extent of COVID-19 community transmission and pre-existing immunity acquired prior to the initiation of the vaccine program is likely to have reduced the power of this study, and may have decreased the incremental estimates of effectiveness of the vaccines evaluated.

Seventy-two countries worldwide currently approve CoronaVac for emergency use (on May 19th of 2022),[13] representing most of the world population. Our study contributes to the increasing literature showing CoronaVac is effective to prevent severe adverse outcomes of Covid-19. A previous study from Arregocés-Castillo et al.[14] in Colombia assessed the effectiveness of COVID-19 vaccines against hospitalization, death after hospitalization, and death without hospitalization in the entire population older than 60 years in the country. They found BNT162b2 reduced death after hospitalization by 94·8% (95% CI, 93·3 to 96·0) in people older than 60 years and 92·7% (95% IC, 85·4 to 96·4); while the CoronaVac effectiveness for this outcome was 72·1% (95% CI, 70·1 to 73·9) in those older than 60 years and 66·3% (95% CI, 63·4 to 69·0) in people over 80 years old. Our study uses data from poor population in northern Colombia, which is different from the Arregocés-Castillo et al. study that used the entire Colombian population. Our results for CoronaVac reported, descriptively, a lower effectiveness than the previous study in the entire Colombian population, while for BNT162b2 we found a higher effectiveness. The causes of these differences are unknown, but several hypotheses are worth exploring. The sample of our study was composed of people with low socioeconomical status, which is a driver of infection and adverse outcomes. Studies from United States,[15,16] Germany,[17] UK,[18] Chile,[19] and Colombia[20] have shown population with lower socioeconomic status have higher infection rate,[16–18,20,21] suggesting our population may have increased infection dynamics and more seroprevalence at the time of the study. A Colombian study[20] showed that during the pandemic, lower socioeconomic status was associated to longer time at work when symptomatic, longer time at work with a known positive contact, longer time working outside home, and longer time between symptoms and test date and test result. A study in the UK also showed how different infection waves were associated to different risks of infection according to socioeconomic status. This increased infection susceptibility could have potentially increase previous immunity among poor population, potentially decreasing the effectiveness of vaccination of CoronaVac in our sample. Another potential cause for these differences is lower statistical power in our sample, with Arregocés-Castillo et al. studying 1.4 million subjects in the unvaccinated cohort. Another difference between our analysis and the previous study is that the Arregocés-Castillo et al. assessment stratified death into two outcomes (with and without hospitalization), with lower effectiveness of death without hospitalization compared to deaths with hospitalizations. Our analyses do not stratify death into these categories, making the differences between studies less marked.

A study from Brazil reported a 50% reduction in symptomatic cases using a matched test-negative case-control study.[4] Another large cohort in Brazil reported CoronaVac had a 73·7% effectiveness against COVID-19 deaths, 73·8% for critical care admission, and 52·7% for infection.[11] In the present study, we did not find a

significant reduction from receiving two doses of CoronaVac in symptomatic cases of confirmed COVID-19. However, we did find a significant reduction in deaths in younger population and more effectiveness for more severe outcomes, suggesting that CoronaVac may have greater efficacy against more severe disease. A report in Chile showed an effectiveness of 67% for symptomatic cases and 77·7% (95% CI, 25–100) for deaths with confirmed COVID-19.[5] Chile vaccinated 90% of its population with CoronaVac,[5] with a strong reduction of daily cases during April and May of 2021.[1] Despite this, Chile reported the largest number of daily cases on June 10th of 2021 since the start of the pandemic.[1] Chilean data might suggest that the effectiveness of CoronaVac is lower for symptomatic cases[4] and waning overtime.[22] The trends and peak of cases after 50% coverage with CoronaVac in Chile also might be explained by the reduced or no protection against infections or symptomatic cases in older population, as the present study shows. These hypotheses need further study.

Post-licensure studies of BNT162b2 have been published for Israel,[23,24] United States,[25,26] and the UK.[27] The present study shows BNT162b2 has strong protection against symptomatic cases, hospitalization, critical care, and deaths. COVID-19 vaccination coverage of fully vaccinated people by June 10th of 2021 was 57% with BNT162b2 in Israel; and 43% in the UK, vaccinating with BNT162b2, Vaxzevria, mRNA-1273, and JNJ-78436735. The 7-day average number of deaths was two for Israel and eight for the UK on June 10th of 2021 (from a previous peak of 65 and 1248, respectively). The reduction of cases was also substantial in these two countries.

Colombia carries out genomic surveillance of SARS-CoV-2 and its variants, albeit with much less intensity than many high-income settings. According to the limited surveillance data available, the Mu variant predominated in the region in between March and August of 2021,[12] with an estimated 60% prevalence of average monthly samples[12] (Figure 1). The Mu variant has shown more resistance to SARS-CoV-2 antibodies than the Beta variant in a recent study.[28] Previous studies show BNT162b2 has effectiveness against Alpha, Beta, and Gamma variants of SARS-CoV-2.[11,29–31] Our study suggests that both BNT162b2 and CoronaVac offer protection against Covid-19 severe outcomes related to the Mu variant as well.

In contrast to BNT162b2, we found a significant increase in symptomatic COVID-19 cases occurring 14 days or more after of the second CoronaVac dose compared to unvaccinated subjected. For this outcome, we cannot eliminate the possibility of a health seeking bias that would increase the frequency of disease among vaccinated. This bias would only have an effect in milder outcomes, such as symptomatic and hospitalized COVID-19, and would be less likely to occur in more severe outcomes that are likely to universally result in health seeking behavior, such as death or critical care admission. Other bias affecting this outcome may be related to testing differences, although by selecting patients with less than ten days between testing and symptoms start did not change the direction of the results in this outcome.

The evaluation of interventions outside the controlled environment of a clinical trial setting is important, especially for mass interventions such as COVID-19 vaccination. Our study compiles and reports data from of a large insurer in Colombia and shows the effect of CoronaVac and BNT162b2 in the field. As with any observational study, ours has limitations and strengths. Our main limitation is related to potential health seeking biases, especially the non-hard outcomes, where the effectiveness was null or significatively negative (i.e., vaccination increased the disease rate for CoronaVac). Another limitation is the potential misclassification of COVID-19 outcomes due to delayed testing, although in our sensitivity analysis, the exclusion of those tested after ten days of symptoms start did not significantly alter the results. The data in the present study only had a mean follow-up of 107 days for CoronaVac, therefore we could not assess waning immunity. The frequency of some comorbidities is relatively low for the older population in present cohort. In the United States, the prevalence of controlled hypertension was 49% in 2015 in the population over 60 years old,[32] and another study in Colombia reported a prevalence of treated hypertension of 40%.[33] One potential cause for these differences is underreporting. Another possible explanation is that our sample comes from low-income backgrounds, therefore potentially increasing the number of subjects with undetected comorbidities. Our results have to be interpreted in the light of these limitations.

Taking the entire evidence into context, the data suggest the effectiveness of two doses of CoronaVac and BNT162b2 to prevent COVID-19 deaths could be substantial in a scenario where Mu predominates. More studies are needed to assess its protection against milder and asymptomatic disease, against other predominant variants, against older population for CoronaVac, and the potential vaccine waning of its effectiveness.

Contributors

Dr. Paternina-Caicedo conceptualized the analysis, wrote the original draft, reviewed, and edited the manuscript, visualized, and made the formal analysis. Dr. Jit aided the methodology, reviewed, and edited the manuscript. Dr. Alvis-Guzman conceptualized the study, contributed to methodology, supervised the research, and reviewed the manuscript. Dr. Fernandez, Mr. Hernandez, and Dr. Paz aided conceptualizing, data curation, project administration, and contributed reviewing the

drafts of the manuscript. Dr. Rojas-Suarez, Dr. Dueñas, and Mr. Alvis-Zakzuk contributed to validation of results, methodology, and reviewing the drafts of the manuscript. Dr. Smith contributed to the design, aided the methodology, and reviewed the drafts of the manuscript. Dr. De la Hoz-Restrepo conceptualized, contributed to the methodology, supervised, and review and edited the draft of the manuscript. All authors reviewed and approved the final manuscript for submission. Dr. Paternina-Caicedo, Dr. Fernandez, and Mr. Hernandez accessed and verified the original data for analysis.

Data sharing statement

Data for this paper will be shared on a reasonable request without identifiers after receiving a signed data-sharing agreement where the researching requesting the data commits, among other things, to the use of the data for research proposes, not to identify any individual, and destroy the data after all analysis are completed.

Declaration of interests

No author declares any conflicts of interest.

Acknowledgements

MJ has received funding from the UK National Institute for Health Research (HPRU-2019-NIHR200908, HPRU-2019-NIHR200929), the European Union's Horizon 2020 research and innovation programme SC1-PHE-CORONAVIRUS-2020 - project EpiPose (No 101003688) and the Bill & Melinda Gates Foundation (INV-016832). No other sources of funding are reported.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.lana.2022.100296.

References

1 Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis*. 2020;20:533–534.
2 Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N Engl J Med*. 2020;383:2603–2615.
3 Baden LR, el Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2021;384:403–416.
4 Hitchings MDT, Ranzani OT, Torres MSS, et al. Effectiveness of CoronaVac among healthcare workers in the setting of high SARS-CoV-2 gamma variant transmission in Manaus, Brazil: a test-negative case-control study. medRxiv 2021; 2021.04.07.21255081.
5 R A. Ministerio de Salud de Chile. Santiago de Chile: Effectiveness of the inactivated CoronaVacvaccine against SARS-CoV-2 in Chile; 2021. https://www.minsal.cl/wp-content/uploads/2021/04/Effectiveness-of-the-inactivated-CoronaVac-vaccine-against-SARS-CoV-2-in-Chile.pdf.
6 Strategic Advisory Group of Experts on Immunization of the World health Organization. Interim Recommendations for Use of the Inactivated COVID-19 Vaccine. CoronaVac, developed by Sinovac; 2021:1.
7 Pan American Health Organization. Colombia Receives the First Vaccines Arriving in the Americas through COVAX - PAHO/WHO | Pan American Health Organization. 2012. published online March 1; https://www.paho.org/en/news/1-3-2021-colombia-receives-first-vaccines-arriving-americas-through-covax. Accessed 29 June 2021.
8 Instituto Nacional de Salud. Noticias coronavirus-genoma. 2022. https://www.ins.gov.co/Noticias/Paginas/coronavirus-genoma.aspx. Accessed 16 May 2022.
9 Ministerio de Salud y Proteccion Social. Vacunación Contra COVID-19. https://www.minsalud.gov.co/salud/publica/Vacunacion/Paginas/Vacunacion-covid-19.aspx. Accessed 29 June 2021.
10 Ministerio de Salud y Proteccion Social. *PAIWEB*. 2021. https://paiweb2.paiweb.gov.co/login. Accessed 29 June 2021.
11 Cerqueira-Silva T, Oliveira V de A, Pescarini J, et al. Influence of age on the effectiveness and duration of protection in Vaxzevria and CoronaVac vaccines. medRxiv 2021; 2021.08.21.21261501.
12 Instituto Nacional de Salud. Instituto Nacional de Salud | Colombia Bienvenido a. 2022; published online Aug 13. http://www.ins.gov.co/Paginas/Inicio.aspx. Accessed 4 Feb 2022.
13 List of COVID-19 vaccine authorizations - Wikipedia. https://en.wikipedia.org/wiki/List_of_COVID-19_vaccine_authorizations#CoronaVac. Accessed 12 July 2021.
14 Arregocés-Castillo L, Fernández-Niño J, Rojas-Botero M, et al. Effectiveness of COVID-19 vaccines in older adults in Colombia: a retrospective, population-based study of the ESPERANZA cohort. *Lancet Healthy Longev*. 2022;3:e242–e252.
15 Dalton JE, Gunzler DD, Jain V, et al. Mechanisms of socioeconomic differences in COVID-19 screening and hospitalizations. *PLoS One*. 2021;16: e0255343.
16 Levy BL, Vachuska K, Subramanian SV, Sampson RJ. Neighborhood socioeconomic inequality based on everyday mobility predicts COVID-19 infection in San Francisco, Seattle, and Wisconsin. *Sci Adv*. 2022;8:(7):eabl3825.
17 Hoebel J, Michalski N, Diercke M, et al. Emerging socio-economic disparities in COVID-19-related deaths during the second pandemic wave in Germany. *Int J Infect Dis*. 2021;113:344–346.
18 Office for National Statistics. Coronavirus (COVID-19) Case Rates by Socio-Demographic Characteristics. England - Office for National Statistics; 2022. published online Feb 14. https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/coronaviruscovid19caseratesbysociodemographiccharacteristicsengland/1september2020to10december2021#socio-economic-factors. Accessed 17 May 2022.
19 Mena GE, Martinez PP, Mahmud AS, Marquet PA, Buckee CO, Santillana M. Socioeconomic status determines COVID-19 incidence and related mortality in Santiago, Chile. *Science*. 2021;372:(6545) eabg5298.
20 Laajaj R, Webb D, Aristizabal D, et al. Understanding how socioeconomic inequalities drive inequalities in COVID-19 infections. *Sci Rep*. 2022;12:1–10.
21 Foster HME, Ho FK, Mair FS, et al. The association between a lifestyle score, socioeconomic status, and COVID-19 outcomes within the UK Biobank cohort. *BMC Infect Dis*. 2022;22:1–13.
22 Sauré D, O'Ryan M, Torres JP, Zuniga M, Santelices E, Basso LJ. Dynamic IgG seropositivity after rollout of CoronaVac and BNT162b2 COVID-19 vaccines in Chile: a sentinel surveillance study. *Lancet Infect Dis*. 2021;22(1):56–63.
23 Haas EJ, Angulo FJ, McLaughlin JM, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. *Lancet North Am Ed*. 2021;397:1819–1829.
24 Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *N Engl J Med*. 2021;384:1412–1423.
25 Britton A, Slifka KMJ, Edens C, et al. Effectiveness of the Pfizer-BioNTech COVID-19 vaccine among residents of two skilled nursing facilities experiencing COVID-19 Outbreaks — Connecticut, December 2020-February 2021. *MMWR Recomm Rep*. 2021;70:396–401.
26 Thompson MG, Burgess JL, Naleway AL, et al. Interim estimates of vaccine effectiveness of BNT162b2 and mRNA-1273 COVID-19 vaccines in preventing SARS-CoV-2 infection among health care personnel, first responders, and other essential and frontline workers

— eight U.S. locations, December 2020–March 2021. *MMWR Morb Mortal Wkly Rep*. 2021;70:495–500.
27 Hall VJ, Foulkes S, Saei A, et al. COVID-19 vaccine coverage in health-care workers in England and effectiveness of BNT162b2 mRNA vaccine against infection (SIREN): a prospective, multicentre, cohort study. *Lancet North Am Ed*. 2021;397:1725–1735.
28 Uriu K, Kimura I, Shirakawa K, et al. Neutralization of the SARS-CoV-2 Mu variant by convalescent and vaccine serum. 2021. https://doi.org/10.1056/NEJMc2114706; published online Nov 3.
29 Emary KRW, Golubchik T, Aley PK, et al. Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial. *Lancet North Am Ed*. 2021;397:1351–1362.
30 Madhi S, Baillie V, Cutland C, et al. Efficacy of the ChAdOx1 nCoV-19 Covid-19 vaccine against the B.1.351 variant. *N Engl J Med*. 2021;384:1885–1898.
31 Abu-Raddad L, Chemaitelly H, Butt A. Effectiveness of the BNT162b2 Covid-19 vaccine against the B.1.1.7 and B.1.351 variants. *N Engl J Med*. 2021;385:187–189.
32 Fryar, CD, Ostchega, Y, Hales, C, Zhang, G, and Kruszon-Moran D. Hypertension prevalence and control among adults: United States, 2015–2016. 2019; 2015–6.
33 Barrera L, Gómez F, Ortega-Lenis D, Corchuelo J, Méndez F. Prevalence, awareness, treatment and control of high blood pressure in the elderly according to the ethnic group. Colombian survey. *Colombia Medica*. 2019;50:115–127.

5.3. Reforço da CoronaVac em idosos eleva em 43 vezes o nível de anticorpos, diz estudo chinês

Um estudo chinês publicado na revista de alto impacto Nature Communications mostrou um aumento no nível de anticorpos após a terceira dose da CoronaVac, tanto em idosos quanto em adultos mais jovens. A pesquisa foi realizada pela Universidade de Fudan, pelos Centros de Controle e Prevenção de Doenças das províncias de Hebei e de Jiangsu, e pela farmacêutica Sinovac.

Os cientistas conduziram dois ensaios clínicos de fase 2 para avaliar a imunogenicidade e a segurança de três doses da CoronaVac em 600 adultos, com idades entre 18 e 59 anos, e em 350 idosos, com 60 anos ou mais. Seis meses após a segunda dose, foram observadas quedas nos títulos de anticorpos, assim como ocorre em todas as vacinas atualmente disponíveis contra a Covid-19.

No grupo de adultos, a dose de reforço elevou os níveis de anticorpos em 33 vezes com a dosagem de 6 µg (para 230,9) e em 21 vezes com a dose de 3 µg (para 143,3). O mesmo padrão foi verificado nos voluntários idosos: a terceira dose fez os anticorpos aumentarem 43 vezes (178,9, com 6 µg do imunizante) e 46 vezes (158,5, com 3 µg).

Além disso, após a terceira dose, os anticorpos neutralizantes foram mantidos por mais tempo do que o observado após a segunda. "A dosagem de 6 µg pareceu induzir maior neutralização e manter níveis mais elevados nos seis meses de acompanhamento após o reforço. Isso implica que vacinas contendo maior teor de antígeno [6µg] podem ser consideradas para programas de imunização de reforço", afirmam os pesquisadores.

Publicado em: 3/6/2022

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-022-30864-w OPEN

Six-month follow-up of a booster dose of CoronaVac in two single-centre phase 2 clinical trials

Qianqian Xin[1,8], Qianhui Wu[2,8], Xinhua Chen[2,8], Bihua Han[3,8], Kai Chu[4,8], Yan Song[5], Hui Jin[6], Panpan Chen[6], Wanying Lu[2], Tuantuan Yang[1], Minjie Li[3,9✉], Yuliang Zhao[3,9✉], Hongxing Pan[4,9✉], Hongjie Yu[2,9✉] & Lin Wang[7,9✉]

Determining the duration of immunity induced by booster doses of CoronaVac is crucial for informing recommendations for booster regimens and adjusting immunization strategies. In two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials, immunogenicity and safety of four immunization regimens are assessed in adults aged 18 to 59 years and one immunization regimen in adults aged 60 years and older, respectively. Serious adverse events occurring within 6 months after booster doses are recorded as pre-specified secondary endpoints, geometric mean titres (GMTs) of neutralising antibodies one year after the 3-dose schedule immunization and 6 months after the booster doses are assessed as pre-specified exploratory endpoints, GMT fold-decreases in neutralization titres are assessed as post-hoc analyses. Neutralising antibody titres decline approximately 4-fold and 2.5-fold from day 28 to day 180 after third doses in adults aged 18–59 years of age and in adults aged 60 years and older, respectively. No safety concerns are identified during the follow-up period. There are increases in the magnitude and duration of humoral response with homologous booster doses of CoronaVac given 8 months after a primary two-dose immunization series, which could prolong protection and contribute to building our wall of population immunity. Trial number: NCT04352608 and NCT04383574.

[1] Sinovac Biotech, Beijing, China. [2] School of Public Health, Fudan University, Key Laboratory of Public Health Safety, Ministry of Education, Shanghai, China. [3] Hebei Provincial Center for Disease Control and Prevention, Shijiazhuang, Hebei, China. [4] Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China. [5] Suining County Center for Disease Control and Prevention, Jiangsu, China. [6] Renqiu Center for Disease Control and Prevention, Hebei, China. [7] Sinovac Life Sciences, Beijing, China. [8] These authors contributed equally: Qianqian Xin, Qianhui Wu, Xinhua Chen, Bihua Han, Kai Chu. [9] These authors jointly supervised this work: Minjie Li, Yuliang Zhao, Hongxing Pan, Hongjie Yu, Lin Wang. ✉email: liminjie507@163.com; yuliang_zh1@163.com; panhongxing@126.com; yhj@fudan.edu.cn; wanglin@sinovac.com

Due in part to waning immunity and diminished protection over time following primary immunisation[1–3], particularly against the Delta (B.1.617.2) variant of SARS-CoV-2, many countries and regions are experiencing surges in COVID-19 cases. Booster doses given at 6–8 months after a primary schedule have been shown to increase neutralisation antibody levels against wild-type virus and reduce the immunity gap between wild-type virus and variants of concern[4,5]. Extended primary immunisation series were recommended by the World Health Organisation[6], especially for those at high risk of severe COVID-19 disease, and booster-dose programmes have been initiated in dozens of countries. With gradual understanding of the epidemiological parameters and immune escape potential of the Omicron variant (B.1.1.529), it is of critical importance to assess the protection and persistence of protection that current COVID-19 vaccines can provide.

Interim study results suggest that rates of confirmed infection and severe illness caused by the Delta variant could be significantly reduced in the short term following booster doses[7,8]. However, no experimental data on the long-term kinetics of neutralisation titres have been reported, even though in-vitro neutralisation titres are important predictors of protection from SARS-CoV-2 variants[9,10]. As CoronaVac is a commonly used vaccine and is contributing to the fight against the pandemic, assessing the duration of immunity following booster-dose administration will be important for improving and updating immunisation strategies. The 3 μg dose is the licensed formulation, and an additional (third) dose is recommended to be offered 6 months after the two-dose primary schedule. We conducted a study to assess immune persistence after a homologous booster dose of CoronaVac given 8 months after the 2nd dose of a two-dose primary immunisation series in two population groups: adults aged 18–59 years and adults aged 60 years or older.

Results

In phase-2 clinical trial among 600 healthy adults aged 18–59 years, 129 (92.8%) of 139 participants from cohort 1a-14d-2m and 126 (96.9%) of 130 participants from cohort 2a-28d-2m completed blood sampling to assess immune persistence for 1 year after dose 3 among those assigned a primary third dose. Separately, 135 participants in cohort 1b-14d-8m (95.7% of the 141 participants assigned a booster dose) and 124 participants in cohort 2b-28d-8m (95.4% of the 130 participants assigned a booster dose) completed blood sampling to assess immune persistence for 6 months after dose 3. In phase-2 clinical trial among a total of 350 healthy adults aged 60 years and older, 283 (93.4%) of 303 participants who received a booster dose from cohort 3-28d-8m completed a 6-month follow-up after dose 3. Supplementary Fig. 1 shows the trial profile. Baseline characteristics of participants are shown in the reports of main findings for these two trials[11–13]. Baseline demographic characteristics of participants who received third doses between the study groups were similar (Supplementary Table 1).

There were 141 minor protocol deviations in cohort 1b-14d-8m and 1 minor protocol deviation in cohort 3-28d-8m that did not result in the exclusion of participants from the analysis, including 141 participants in cohort 1b-14d-8m who were given third doses 9–11 days outside of the pre-specified time window, and 1 participant in cohort 3-28d-8m who was given a second dose 5 days outside of the pre-specified time window (Supplementary Fig. 1).

Compared to antibody concentrations on day 28 after the booster dose, neutralization titer declined 3–4-fold by 6 months after the booster dose, which was given 8 months after a two-dose primary vaccination regimen in adults aged 18–59 years. In the 3 μg group in cohort 2b-28d-8m, GMTs decreased from 143.3 (95% CI 112.3–182.8) on day 28 to 36.4 (95% CI 28.7–46.1) on day 180 after a booster dose (Fig. 1 and Supplementary Fig. 2, Supplementary

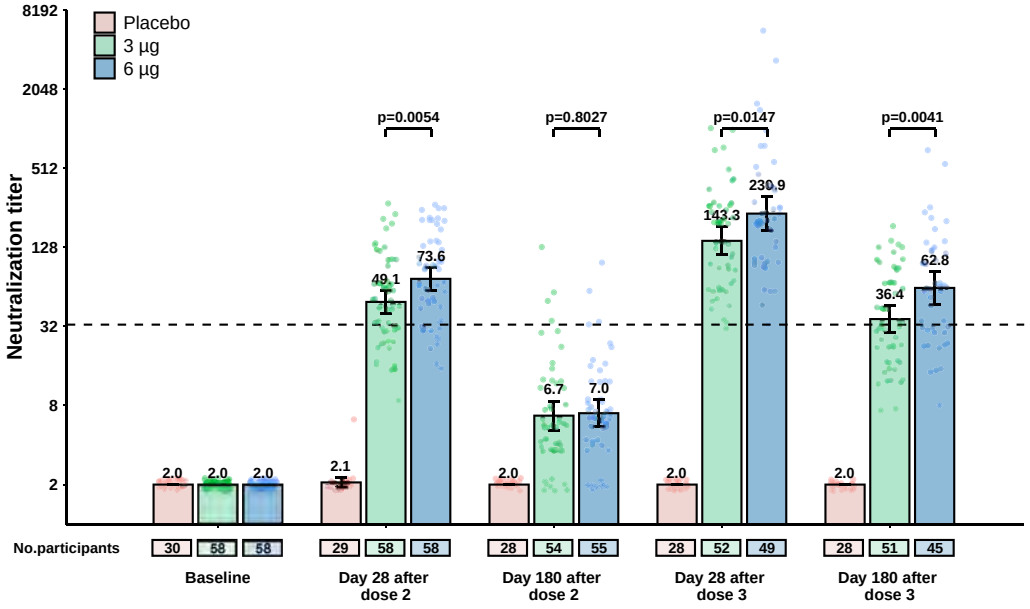


Fig. 1 Neutralising antibody levels to ancestral SARS-CoV-2 in cohort 2b-28d-8m (adults aged 18–59 years old). The number of participants for each group (placebo group, pink; 3 μg group, green; 6 μg group, blue) at each visit included in the analysis is provided below the bars. Dots are reciprocal neutralising antibody titres for individuals in the per-protocol population. Numbers above the bars are geometric mean titres (GMTs), and error bars indicate 95% CIs. GMTs and corresponding 95% CIs were calculated on the basis of standard normal distributions of log-transformed antibody titres. Numbers above the short horizontal lines are p values for comparisons between 3 μg group and 6 μg group using group *t* tests with log-transformation (two-sided). Titres lower than the limit of detection (1:4) are presented as half the limit of detection. The dotted horizontal line represents the protective threshold (1:33).

Table 2). With the exception of baseline and day 180 after dose 2, GMTs at other timepoints in cohort 2b-28d-8m were significantly higher in the 6 μg group than in the 3 μg group (Supplementary Fig. 2 and Supplementary Table 2). GMTs decreased from 137.9 (95% CI 99.9–190.4) on day 14 to 33.4 (95% CI 25.0–44.6) on day 180 after booster doses in cohort 1b-14d-8m (Fig. 1, Supplementary Fig. 2 and Supplementary Table 2). GMTs in cohort 1b-14d-8m on day 180 after the booster dose were significantly higher ($P = 0.02$) in the 6 μg group than in the 3 μg group; there were no significant differences between the two-dose amounts at other timepoints (Supplementary Fig. 2 and Supplementary Table 2). Regardless of the interval between the first two doses and antigen amount, by 1 year after a primary third dose, GMTs in vaccination groups were all at least twofold above the detection limit in cohort 1a-14d-2m and cohort 2a-28d-2m. There were no significant differences in GMTs between the 3 μg groups and the 6 μg groups at 1 year after dose 3 in the two cohorts (Supplementary Fig. 2 and Supplementary Table 2).

A similar pattern was observed in cohort 3-28d-8m, in which neutralisation titres declined from 158.5 [95% CI 96.9–259.1] on day 28 to 53.2 [95% CI 39.7–71.1] on day 180. GMTs on day 180 after the booster dose were highest in the 6 μg group (GMT 91.2 [95% CI 71.5–116.3], $P < 0.0001$), followed by the 3 μg group (Fig. 2 and Supplementary Table 3). In the 3 μg group and the 6 μg group, GMTs 6 months after booster doses among older adults (60 years and older) were numerically higher than among younger adults (18–59 years old), but without a statistical difference ($P = 0.05$). Results of sensitivity analyses showed that the use of average dilutions has no significant impact on the values of neutralisation antibody titre (Supplementary Tables 4–7).

GMT fold decreases during the 6 months after primary two doses, primary three doses, and booster doses were compared among vaccination groups, calculated as the ratio of GMT on day 28 to GMT on day 180 after the specific dose. Taking the 3 μg group in cohort 2b-28d-8m as an example, the GMT fold decrease between day 28 and day 180 after a booster dose (4.1-fold) was significantly lower than that observed between day 28 and day 180 after the second dose (6.8-fold; $P = 0.0007$; Fig. 3), which was numerically lower than that of day 28 and day 180 after primary three doses in cohort 2a-28d-2m (4.9-fold; $P = 0.35$; Supplementary Fig. 3). GMT fold decreases between day 28 and day 180 after the second dose were similar in cohort 1b-14d-8m (7.3-fold) and cohort 2b-28d-8m (6.8-fold; $P = 0.75$; Supplementary Fig. 3), regardless of the interval of first two doses. Likewise, the GMT fold decrease between day 28 and day 180 after the booster dose (2.5-fold) was significantly lower than that between day 28 and day 180 after the second dose (10.7-fold; $P < 0.0001$; Fig. 3). Compared with adults aged 18–59 years old (cohort 2b-28d-8m), the GMT fold decrease was greater in adults aged 60 years and older (cohort 3-28d-8m) after primary two doses (6.8-fold vs 10.7-fold, $P = 0.03$), but was lower after booster doses (4.1-fold vs 2.5-fold, $P < 0.0001$; Fig. 3). There were no significant differences in GMT fold decreases between the 3 μg groups and 6 μg groups after booster doses among all the vaccination groups, irrespective of vaccination schedules and age grouping (Fig. 3, Supplementary Fig. 3 and Supplementary Fig. 4).

Serious adverse events that occurred from the beginning of immunisation to 6 months after second doses in cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m, and that occurred from the beginning of immunisation to 6 months after third doses in cohort 1a-14d-2m and cohort 2a-28d-2m have been reported previously[13]. During the 6-month follow-up after booster doses in cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m, serious adverse events were reported in one (2%) of 52 participants in the 3 μg group in cohort 2b-28d-8m (Supplementary Table 8), in four (5%) of 85 participants in the

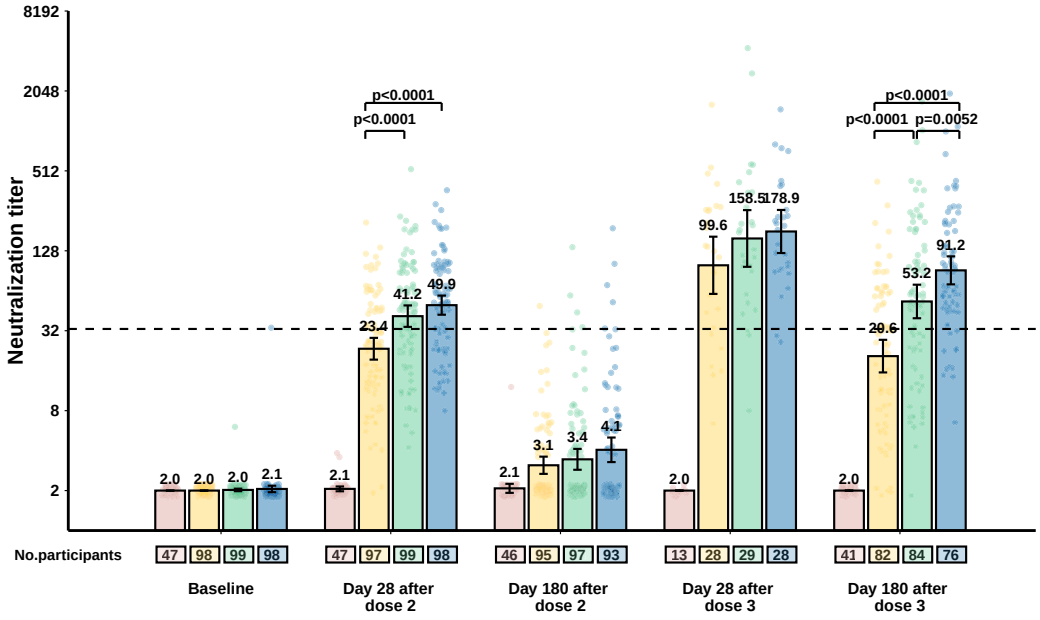


Fig. 2 Neutralising antibody levels to ancestral SARS-CoV-2 in cohort 3-28d-8m (adults aged 60 years and older). The number of participants for each group (placebo group, pink; 1.5 μg group, yellow; 3 μg group, green; 6 μg group, blue) at each visit included in the analysis is provided below the bars. Dots are reciprocal neutralising antibody titres for individuals in the per-protocol population. Numbers above the bars are geometric mean titres (GMTs), and error bars indicate 95% CIs. GMTs and corresponding 95% CIs were calculated on the basis of standard normal distributions of log-transformed antibody titres. Numbers above the short horizontal lines are *p* values for comparisons between the 1.5 μg group, the 3 μg group and the 6 μg group using ANOVA models with log-transformation. Bonferroni correction done as a post hoc test if the variance was significant. Only *P* values indicating significant differences are marked. Titres lower than the limit of detection (1:4) are presented as half the limit of detection. The dotted horizontal line represents the protective threshold (1:33).

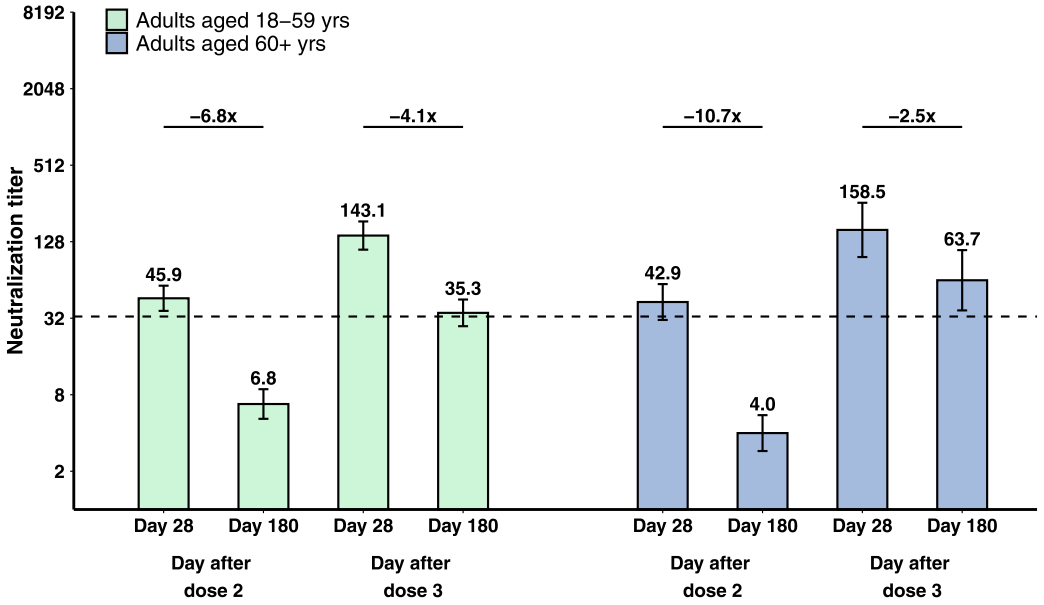


Fig. 3 Decline in neutralising antibodies to ancestral SARS-CoV-2 in 3 μg groups in cohort 2b-28d-8m and cohort 3-28d-8m. The number of participants with paired samples for adults aged 18–59 (green) and adults aged 60 and over (blue) was 49 and 29, respectively. Numbers above the bars are geometric mean titres (GMTs), and error bars indicate the 95% CIs. The dotted horizontal line represents the protective threshold (1:33). Numbers above the short horizontal lines are pairwise fold-change values. GMTs and corresponding 95% CIs were calculated on the basis of standard normal distributions of log-transformed antibody titres. GMT fold decreases in neutralisation titre were calculated as ratios of paired sera at two visits. Comparisons between groups were conducted by group *t* tests with log-transformation (two-sided). *P* values of pairwise comparisons were $P < 0.0001$, $P < 0.0001$, $P < 0.0001$, $P = 0.0187$, from left to right, respectively.

1.5 μg group, in five (6%) of 90 in the 3 μg group, in three (4%) of 81 in the 6 μg group, and in two (4%) of 47 in the placebo group in cohort 3-28d-8m (Supplementary Table 9). No participant in cohort 1b-14d-8m reported a serious adverse event. No serious adverse event in either trial was considered by the investigators to be related to vaccination, and no pre-specified trial-halting rules were met.

Discussion

Following a primary three-dose regimen for immunisation, neutralisation antibody levels declined 6 months later and remained stable during the next 6 months. Neutralisation antibody levels were substantially increased by booster doses given 8 months after primary two-dose regimens and were maintained over the following 6 months—comparable with levels after primary two-dose immunisation regimens. When booster doses were given 8-month after primary immunisation, the decay rates of neutralisation titres over the 6 months after booster-dose administration were much slower than that after primary two-dose regimens regardless of age group and antigen amount. Memory B cells are known to proliferate and produce antibodies that maintain immunity after repeated exposure to antigens—a phenomenon that likely maintains protection and contributes to building our wall of population immunity.

Observed GMT fold decreases during the 6 months following primary two doses in the two age groups in our CoronaVac study were in line with results of a BNT162b2[4] vaccine study, which were 6.0-fold in 18–55 years of age and 13.1-fold in 65–85 years of age from 7 days after dose 2 to before dose 3 (7.9–8.8 months after dose 2). We found that GMT fold decreases after booster doses were lower and neutralisation titres 6 months after booster doses were numerically higher in adults aged 60 years and older (cohort 3-28d-8m) than in adults aged 18–59 years old (cohort 2b-28d-8m), which is in contrast with common sense that immune responses to vaccination are generally weaker in older adults[14]. Notably, differences were small and there was overlap between younger adults and older adults in neutralising activity against SARS-CoV-2 viruses in the mRNA-1273 vaccine recipients[15]. Age did not appear to compromise antibody response, even after accounting for severity among COVID-19 patients[16]. More experimental data are required to address the age heterogeneity of long-term neutralisation dynamics following vaccination.

A meta-analysis that summarised immune escape potential of different SARS-CoV-2 variants against immunity induced by both natural infection and vaccination showed that the average fold reduction of neutralising antibody level against the Delta variant was 2.4 (95% CI: 1.1–5.2) for inactivated vaccines in live virus neutralisation assays when compared to that of prototype strains[17]. However, for individuals vaccinated with CoronaVac, the average reduction against Delta was 9.2-fold compared with the prototype strain using authentic virus neutralisation assay[18]. Currently, there are no available data for immune evasion of the humoral immunity elicited by inactivated vaccines for the recently emerged Omicron variant. One report showed that sera from individuals who received two doses of BNT162b2 exhibited an average 25-fold reduction in neutralisation titres against the Omicron variant compared to wild-type virus when using a pseudovirus neutralisation test[19]. Another study showed a higher fold reduction of 41.4 for the Omicron variant among individuals with previous infection or vaccination[20].

Even though neutralisation titres induced by COVID-19 vaccines decline over time and against variants, vaccine effectiveness against severe COVID-19 illness is sustained, including against severe outcomes caused by Delta[21]. Although the limited available evidence shows that the immune escape of Omicron is significant, vaccine effectiveness against hospitalisation may be well maintained. Booster vaccination with current vaccines increases the

affinity of antibody and neutralisation potency better than that achieved with primary vaccination only, and this effect can likely be predicted to provide robust protection from severe infection outcomes from the current SARS-CoV-2 variants of concern[9]. One recent study reported that a moderate to high vaccine effectiveness against mild infection of 70–75% was seen in the early period after a booster dose of BNT162b2 following either ChAdOx1-S or BNT162b2 as a primary series, despite the longer intervals after primary vaccination[22], underscoring the necessity of timely administration of a booster dose.

Higher antigen content appeared to induce higher neutralisation titres and maintain higher levels in the 6 months of follow-up after booster doses in the medium term. This implies that vaccines containing higher antigen content (i.e. 6 μg) could be considered for booster immunisation programmes. Heterologous booster vaccination has been shown to induce strong humoral responses and augment neutralisation potency[23,24]. At 4–8 months after primary immunisation with CoronaVac, a significantly higher degree of humoral immunogenicity against the prototype strain and the Gamma, Beta, and Delta variants was observed following a third dose of ZF2001 (a protein subunit vaccine manufactured by Anhui Zhifei Longcom Biopharmaceutical)[18]. A heterologous prime-boost regimen with Convidecia (a type-5-adenovirus-vectored COVID-19 vaccine manufactured by CanSino) after priming with CoronaVac 3–6 months earlier induced approximately 5.9-fold higher live virus neutralising antibodies than homologous boosting induced[25]. By identifying various forms of antigens from vaccines made on different platforms, the immune system apparently can be trained to produce a more balanced and comprehensive immune response that enhances the effect of current vaccines through heterologous immunisation strategies. Heterologous boosting has clear policy implications, as it can provide solutions to curb the pandemic of emerging variants before developing new vaccines. It should be noted there are no large-scale heterologous immunisation practices until recently, and more high-quality safety and effectiveness research evidence is required to improve immunisation strategies. In addition, several research studies have shown that extended dosing intervals generate more favourable immune responses[5,26]. "Mix-and-match" regimens and longer dosing interval strategies may also be helpful in lower-income countries, where some vaccines may be in short supply some of the time. With much of the world yet to be vaccinated, re-doubling our efforts for equitable and speedy vaccine delivery on a global scale and improving initial vaccination coverage should be our primary focus.

Immune memory is what leads to long-term immunity, but it is difficult to predict how long immunity will last because the exact mechanisms of protective immunity against SARS-CoV-2 or COVID-19 are still not clear. The 6-month marker in our study is an important milestone, but long-term immune response and effectiveness need to be continuously monitored into the foreseeable future.

Our study has several limitations. First, T-cell responses and neutralisation tests in vitro against emerging variants were not assessed in our study; these should be further explored. Second, multicentre studies will be needed to assess primary outcomes among subpopulations for whom our study had relatively small proportions, for example, people with multiple underlying conditions or immunosuppressive conditions. Third, the follow-up time of our study is relatively short. However, timely reporting of follow-up data is very important for ongoing adjustment of immunisation strategies in the context of a pandemic with frequent emergence of variants. Fourth, although neutralising antibodies are related to protection, actual protection from infection with current and future variants will need to be monitored with real-world observational studies. Further research to identify correlates of protection is essential.

In conclusion, a homologous booster dose of CoronaVac given 8 months after 2nd dose of the primary two-dose immunisation recalls robust neutralisation antibody levels and significantly delays antibody attenuation in adults aged 18 years and older. More experimental and long-term monitoring data are needed to optimise the selection of booster doses and booster-dose intervals to most effectively combat the pandemic.

Methods

Study design and participants. The study designs and methods for these two phase II trials have been previously reported[11]. Key exclusion criteria for trial enrolment included suspected or laboratory-confirmed SARS-CoV-2 infections and known allergy to any vaccine component. A complete list of exclusion criteria is in the protocol in Supplementary Material. All participants gave written informed consent to participate in the study before administration of first doses and booster doses. The two trials were registered with ClinicalTrials.gov, NCT04352608 and NCT04383574.

Briefly, the initial trial involving 600 healthy adults aged 18–59 years old in a single-centre, double-blind, randomised, placebo-controlled, phase-2 clinical trial conducted from May 3, 2020 in Suining county, Jiangsu province, China. Following enrollment, participants were randomised to receive three doses of either 3 μg of CoronaVac, 6 μg of CoronaVac, or placebo with an interval of 14 days or 28 days between the first two doses and 2 months or 8 months between the second and third doses; the respective study groups were cohort 1a-14d-2m, cohort 1b-14d-8m, cohort 2a-28d-2m, and cohort 2b-28d-8m. One hundred fifty participants were assigned to each cohort, and 3 μg or 6 μg of CoronaVac or placebo were randomly assigned in a 2:1:1 allocation ratio.

The other trial, involving 350 healthy adults aged 60 years and older, was a single-centre, double-blind, randomised, placebo-controlled, phase-2 clinical trial conducted from June 12, 2020 in Renqiu county, Hebei province, China. Following enrollment, participants were randomised to receive three doses of 1.5, 3 or 6 μg of CoronaVac or placebo with an interval of 28 days between the first two doses and 8 months between second and third doses; this study group is cohort 3-28d-8m. Randomisation was performed with a 2:2:2:1 allocation ratio.

Electronic Data Capture (EDC) RIEHEN (Version: 2.1.1608) was used to establish the electronic Case Report Form (eCRF) in both trials to record clinical trial data. Information was inputted with standard language according to the EDC instructions and eCRF filling instructions. Randomisation codes for each vaccination schedule cohort were generated individually and randomly assigned using block randomisation developed with SAS version 9.4. Adults aged 18–59 years were assigned with a block size of five and adults aged 60 years and older were assigned with a block size of fourteen. Concealed random group allocations and blinding codes were kept in signed and sealed envelopes. Investigators, participants, and laboratory staff were masked to group assignment. The randomisation code was assigned to each participant in sequence in the order of enrolment by investigators, who were involved in the rest of the trial.

Follow-up. Essential steps and timing for each visit specified in the protocol are shown in Supplementary Visit Plan. Conditions leading to participant withdrawal and suspension criteria were reported previously[11], including unacceptable adverse events, abnormal clinical manifestations, participants' request. Participants who received primary third doses 2 months after the second dose (cohort 1a-14d-2m and cohort 2a-28d-2m) had blood samples drawn 1 year after the third dose to evaluate immune persistence of this three-dose primary immunisation regimen. Participants who received booster doses 8 months after the second dose (cohort 1b-14d-8m, cohort 2b-28d-8m and cohort 3-28d-8m), had blood samples drawn 6 months after the booster dose to evaluate immune persistence of this booster regimen.

Immunological assessment methods and related procedures are described in the Supplementary Neutralisation Assay. Neutralising antibodies against infectious SARS-CoV-2 (virus strain SARS-CoV-2/human/CHN/CN1/2020, GenBank accession number MT407649.1, https://www.ncbi.nlm.nih.gov/nuccore/MT407649.1) were quantified using a microcytopathogenic effect assay. We treated the neutralising antibody titer of the serum specimen as the reciprocal of the average dilutions of two wells when one of two adjacent wells was pathological while the other not. To avoid the use of the average would not deflate or inflate the values of neutralisation antibody titre, we conducted sensitivity analyses only to adopt higher dilutions or lower dilutions respectively. Serious adverse events were recorded for 6 months after the third dose for participants in every cohort. Serious adverse events were coded by the Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class. The existence of causal associations between adverse events and vaccination was determined by the investigators.

Outcomes. A complete list of study endpoints is provided in the Supplementary Study Endpoints. Results as of 28 days after booster doses (for cohort 1b-14d-8m, cohort 2b-28d-8m and cohort 3-28d-8m) and 6 months after primary three doses (for cohort 1a-14d-2m and cohort 2a-28d-2m) have been reported previously[11]. Here, we report the follow-up immunogenic results including geometric mean titres (GMTs) of neutralising antibodies to infectious SARS-CoV-2 one year after

the full schedule immunisation (for cohort 1a-14d-2m and cohort 2a-28d-2m), 6 months after the booster dose (for cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m), all of which are pre-specified exploratory endpoints. As did Khoury and colleagues[10], we used a protective threshold of 33 for CoronaVac vaccine, which was defined as the neutralisation titer at which an individual will have a 50% protective efficacy for CoronaVac. Titres lower than the limit of detection (1:4) were treated as half the limit of detection.

Serious adverse events occurring within 6 months after booster doses (for cohort 1b-14d-8m, cohort 2b-28d-8m and cohort 3-28d-8m) were recorded; serious adverse events were pre-specified secondary endpoints. Comparisons of GMT fold decreases in neutralisation titres within 1 year after full-course vaccination for cohort 1a-14d-2m and cohort 2a-28d-2m, and within 6 months after second doses and third doses for cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m, were post hoc analyses. Given that the 3 μg dose is the licensed formulation and an additional (third) dose is recommended to be offered 6 months after the two-dose primary schedule, we present results for the 3 μg groups in cohort 2b-28d-8m and cohort 3-28d-8m in the main text and provide detailed results for other intervention groups in the Supplementary.

Ethical statement. We complied with all relevant ethical rules. The complete study protocol for adults aged 18–59 years old was approved by the ethics committees of Jiangsu Provincial Centre for Disease Control and Prevention (JSJK2020-A021-02), and the complete study protocol for adults aged 60 years and older was approved by the ethics committees of Hebei Provincial Centre for Disease Control and Prevention (IRB2020-006).

Statistical analysis. The sample size was determined following requirements of the National Medical Products Administration, China's regulatory authority for vaccines. We assessed immunological endpoints in the per-protocol population, which included all participants who completed their assigned doses and had antibody results available according to the protocol. Serious adverse events were evaluated in the safety population for booster-dose groups, which included all participants who received a booster dose of the study vaccine. GMT fold decreases in neutralisation titres were assessed among participants who received three doses and had antibody results from all visits.

Pearson χ^2 test or Fisher's exact test were used to analyse categorical outcomes. We calculated 95% CIs for categorical outcomes using the Clopper–Pearson method.

We calculated GMTs and corresponding 95% CIs on the basis of standard normal distributions of log-transformed antibody titres. GMT fold decreases in neutralisation titre were calculated as ratios of paired sera at two visits. ANOVA models with log-transformation were used to detect differences among groups. Comparisons were done between groups by group t tests with log-transformation and Bonferroni correction done as a post hoc test if the variance was significant. Hypothesis testing was two-sided, and we considered P values of less than 0.05 to be significant. We used R software version 4.0.2 for all analyses.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The study protocols are available in the Supplementary Material. To protect participants' confidentiality, the individual participant data that underlie the results reported in this article (text, tables, figures and extended data) will only be shared after de-identification. Due to the clinical trial in adults aged 60 years and older is ongoing, in order to maintain the blind status of this trial, the data will be available following clinical study report (CSR) of immune persistence analysis (September 2022). Researchers who provide a scientifically sound proposal will be allowed access to the individual participant data. Proposals should be directed to wanglin@sinovac.com.

Code availability

The R code for the main analysis is available on GitHub at https://github.com/cxhhhh24/sinoVac_antibody.

Received: 24 January 2022; Accepted: 24 May 2022;
Published online: 03 June 2022

References

1. Levin, E. G. et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. *N. Engl. J. Med.* **385**, e84 (2021).
2. Chemaitelly, H. et al. Waning of BNT162b2 vaccine protection against SARS-CoV-2 infection in Qatar. *N. Engl. J. Med.* **385**, e83 (2021).
3. Cohn, B. A., Cirillo, P. M., Murphy, C. C., Krigbaum, N. Y. & Wallace, A. W. SARS-CoV-2 vaccine protection and deaths among US veterans during 2021. *Science* **375**, 331–336 (2021).
4. Falsey, A. R. et al. SARS-CoV-2 neutralization with BNT162b2 vaccine dose 3. *N. Engl. J. Med.* **385**, 1627–1629 (2021).
5. Flaxman, A. et al. Reactogenicity and immunogenicity after a late second dose or a third dose of ChAdOx1 nCoV-19 in the UK: a substudy of two randomised controlled trials (COV001 and COV002). *Lancet* **398** 981–990 https://doi.org/10.1016/S0140-6736(21)01699-8 (2021).
6. World Health Organization. Highlights from the Meeting of the Strategic Advisory Group of Experts (SAGE) on Immunization. https://cdn.who.int/media/docs/default-source/immunization/sage/2021/october/sage_oct2021_meetinghighlights.pdf?sfvrsn=3dcae610_15 (2021).
7. Barda, N. et al. Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for preventing severe outcomes in Israel: an observational study. *Lancet* **398**, 2093–100. (2021).
8. Bar-On, Y. M. et al. Protection of BNT162b2 vaccine booster against Covid-19 in Israel. *N. Engl. J. Med.* **385**, 1393–1400 (2021).
9. Cromer, D. et al. Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis. *Lancet Microbe* **3**, e52–e61 (2021).
10. Khoury, D. S. et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* **27**, 1205–11. (2021).
11. Zeng, G. et al. Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials. *Lancet Infect. Dis.* **22**, 483–495 (2021).
12. Zhang, Y. et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect. Dis.* **21**, 181–92. (2021).
13. Wu, Z. et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect. Dis* **21**, 803–812 https://doi.org/10.1016/S1473-3099(20)30987-7 (2021).
14. Ciabattini, A. et al. Vaccination in the elderly: the challenge of immune changes with aging. *Semin Immunol.* **40**, 83–94 (2018).
15. Pegu, A. et al. Durability of mRNA-1273 vaccine–induced antibodies against SARS-CoV-2 variants. *Science* **373**, 1372–1377 (2021).
16. Lau, E. H. Y. et al. Neutralizing antibody titres in SARS-CoV-2 infections. *Nat. Commun.* **12**, 63 (2021).
17. Chen, X. et al. Neutralizing antibodies against SARS-CoV-2 variants induced by natural infection or vaccination: a systematic review and pooled meta-analysis. *Clin. Infect. Dis,* **74**, 734–742 https://doi.org/10.1093/cid/ciab646 (2022).
18. Cao, Y. et al. Humoral immunogenicity and reactogenicity of CoronaVac or ZF2001 booster after two doses of inactivated vaccine. *Cell Res.* **32**, 107–109 (2021).
19. Pfizer and BioNTech provide update on omicron variant. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-biontech-provide-update-omicron-variant (2021).
20. Cele, S. et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. *Nature* **602**, 654–656 https://doi.org/10.1038/s41586-021-04387-1 (2022).
21. Krause, P. R. et al. Considerations in boosting COVID-19 vaccine immune responses. *Lancet* **398**, 1377–1380 (2021).
22. Andrews, N. et al. Effectiveness of COVID-19 vaccines against the Omicron (B.1.1.529) variant of concern. *N Engl J Med*. **386**, 1532–1546 https://doi.org/10.1056/NEJMoa2119451 (2022).
23. Chiu, N. C. et al. To mix or not to mix? A rapid systematic review of heterologous prime-boost covid-19 vaccination. *Expert Rev. Vaccines* **20**, 1211–1220 (2021).
24. Reynolds, C. J. et al. Heterologous infection and vaccination shapes immunity against SARS-CoV-2 variants. *Science* **375**, eabm0811 (2021).
25. Li, J et al. Heterologous AD5-nCOV plus CoronaVac versus homologous CoronaVac vaccination: a randomized phase 4 trial. *Nat Med.* **28**, 401–409 https://doi.org/10.1038/s41591-021-01677-z (2022).
26. Payne, R. P. et al. Immunogenicity of standard and extended dosing intervals of BNT162b2 mRNA vaccine. *Cell* **184**, 5699–714 e11 (2021).

Acknowledgements

We thank Dr. Lance Rodewald from the Chinese Center for Disease Control and Prevention for his comments and English language editing. The study was supported by grants from

National Key Research and Development Program (2020YFC0849600), Beijing Science and Technology Program (Z201100005420023), and Key Program of the National Natural Science Foundation of China (82130093). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Author contributions

Q.X., Q.W., X.C., B.H., K.C., M.L., Y.Z., H.P., H.Y. and L.W. formulated the study design, and performed the data collection, analysis, interpretation and writing of the manuscript. H.J., P.C. and Y.S. collected the data and revised the manuscript. T.Y. carried out the laboratory assays and revised the manuscript. W.L. analysed the data and revised the manuscript. All authors had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests

H.Y. has received research funding from Sanofi Pasteur, GlaxoSmithKline, Yichang HEC Changjiang Pharmaceutical Company, and Shanghai Roche Pharmaceutical Company. None of those research funding is related to the development of COVID-19 vaccines. Q.X. and T.Y. were the employees of Sinovac Biotech Ltd., L.W. was an employee of Sinovac Life Sciences Co., Ltd. The remaining authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-30864-w.

Correspondence and requests for materials should be addressed to Minjie Li, Yuliang Zhao, Hongxing Pan, Hongjie Yu or Lin Wang.

Peer review information *Nature Communications* thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

5.4. CoronaVac protege os idosos contra a Covid-19 grave, evidencia estudo chinês

Um estudo chinês realizado em Nanjing mostrou mais uma vez a eficácia da CoronaVac em evitar que idosos desenvolvam a forma grave da Covid-19, demonstrando que a vacina é capaz de proteger inclusive contra a variante delta do SARS-CoV-2. O trabalho foi publicado na revista Aging e conduzido por pesquisadores do Hospital da Universidade Médica de Nanjing, na China.

Os cientistas selecionaram 181 pacientes com 60 anos ou mais que foram infectados pela variante delta, admitidos entre julho e setembro de 2021 no Centro de Saúde Pública de Nanjing. Destes, 111 tinham comorbidades. Os voluntários foram divididos em três grupos: grupo A, com 113 participantes, que não tinha sido imunizado; grupo B, com 46, que havia tomado apenas a primeira dose da CoronaVac; e o grupo C, com 22, que recebeu o esquema completo de duas doses.

Entre os pacientes, 145 foram classificados como casos moderados de Covid-19, 21 evoluíram para a forma grave e 15 foram casos críticos, que resultaram em dois óbitos. Todos os casos críticos ocorreram no grupo A (não vacinado). Esse público também concentrou todos os casos de disfunção de múltiplos órgãos (14), de choque séptico (12) e de Síndrome Respiratória Aguda Grave (15).

Além disso, o estudo apontou que os níveis de anticorpos IgM e IgG encontrados nos pacientes vacinados foram significativamente maiores do que naqueles não imunizados. "Esta pesquisa clínica confirmou que a vacina inativada para SARS-CoV-2 foi eficaz para evitar a gravidade da doença em pacientes idosos com infecção da variante delta, especialmente naqueles com comorbidades", afirmam os autores no artigo.

Publicado em: 18/5/2022

www.aging-us.com AGING 2022, Vol. 14, No. 10

Research Paper

The roles of inactivated vaccines in older patients with infection of Delta variant in Nanjing, China

Xiao-Chun Song[1,*], Xue-Hui Zhou[1,*], Jing-Hui Cheng[1,*], Wen-Hao Zhang[1], Xiao Shen[1], Huan Xu[1], Shuai Nie[1], Ji-Lai Xiao[1], Fang Sun[1], Chang Shu[1], Jiu-Dong Chen[1], Yan Tang[1], Xiang Wang[1], Xin-Pei Sun[2,&], Jia-Kui Sun[1], Ping Feng[1], Qian-Kun Shi[1]

[1]Department of Critical Care Medicine, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, Jiangsu Province, China
[2]Department of General Office, Productivity Center of Jiangsu Province, Nanjing 210042, Jiangsu Province, China
[*]Equal contribution

Correspondence to: Xiang Wang, Xin-Pei Sun, Jia-Kui Sun; **email:** njdrwx2016@163.com, https://orcid.org/0000-0002-0228-0152; sjk0935119@163.com, https://orcid.org/0000-0003-0896-1511; njdrsjk2020@njmu.edu.cn
Keywords: inactivated vaccines, organ dysfunction, older patients, Delta variant, COVID-19
Received: November 25, 2021 **Accepted:** May 7, 2022 **Published:** May 18. 2022

ABSTRACT

Background: The coronavirus disease 2019 (COVID-19) is spreading around the world. The COVID-19 vaccines may improve concerns about the pandemic. However, the roles of inactivated vaccines in older patients (aged ≥60 years) with infection of Delta variant were less studied.

Methods: We classified the older patients with infection of Delta variant into three groups based on the vaccination status: no vaccination (group A, *n* = 113), one dose of vaccination (group B, *n* = 46), and two doses of vaccination (group C, *n* = 22). Two inactivated COVID-19 vaccines (BBIBP-CorV or CoronaVac) were evaluated in this study. The demographic data, laboratory parameters, and clinical severity were recorded.

Results: A total of 181 older patients with infection of Delta variant were enrolled. 111 (61.3%) patients had one or more co-morbidities. The days of "turn negative" and hospital stay in Group C were lower than those in the other groups (*P* < 0.05). The incidences of multiple organ dysfunction syndrome (MODS), septic shock, acute respiratory distress syndrome (ARDS), acute kidney injury, and cardiac injury in Group A were higher than those in the other groups (*P* < 0.05). The MV-free days and ICU-free days during 28 days in Group A were also lower than those in the other groups (*P* < 0.05). In patients with co-morbidities, vaccinated cases had lower incidences of MODS (*P* = 0.015), septic shock (*P* = 0.015), and ARDS (*P* = 0.008).

Conclusions: The inactivated COVID-19 vaccines were effective in improving the clinical severity of older patients with infection of Delta variant.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) continues to spread throughout all parts of the world [1–3], and more and more mutant variants have exacerbated the global pandemic [2, 4], which may also facilitate escape from vaccine protection and current therapies in unexpected ways [5, 6].

In addition to the nonpharmaceutical interventions and symptomatic treatments, new SARS-CoV-2 vaccines may improve concerns about the global pandemic [7]. Several inactivated vaccines against SARS-CoV-2 (e.g., ZF2001, CoronaVac, BBIBP-CorV) have been demonstrated to be generally effective and safe in large sample clinical studies [7–9]. Besides that, these vaccines are also well-tolerated

in 60 years and older adults and could reduce the severity of COVID-19 [7, 10]. However, elderly people with co-morbidities or frailty were usually not included in the previous phase 1 to 3 trials [11]. In both CD8 and CD4 cells, aging has been shown to result in a reduction of T cell receptor diversity, which may lead to reduced T cell survival [11]. Aging could also decrease the production of functional antibodies because of reduced expression of select proteins [11]. Hence, the current vaccines may be theoretically ineffective in older people.

An imported COVID-19 infection related to the Delta strain (the B.1.617.2 variant) erupted in the Chinese city of Nanjing on July 21, 2021 [3, 12]. Considering the mutating variants, the effectiveness of various types of vaccines should also be confirmed by more studies. While conducting our clinical effort to combat the COVID-19 epidemic in Nanjing, we discovered that there were a number of older and vaccinated patients among confirmed cases. Therefore, we aimed to investigate the roles of inactivated SARS-CoV-2 vaccines in older patients with infection of Delta variant, especially in those with co-morbidities.

METHODS

Patients

From July 21 to September 13, 2021, older patients (age ≥60 years) with confirmed infection of Delta variant admitted to specialized isolation units, Nanjing Public Health Center (Nanjing Second Hospital), were recruited to participate in this clinical retrospective study. The only hospital in Nanjing that treated COVID-19 patients was the Nanjing Public Health Center. All the older patients were classified as high-risk groups for severe or critical [12]. Therefore, these patients received grade one (in the ward) or special (in ICU) nursing care in our specialized isolation units. Our institutional review board waived written informed consent since this was retrospective research that gathered de-identified data with no possible danger to the patients. The COVID-19 (Delta variant) was diagnosed in accordance with the guidelines of the National Health Commission (NHC) of China and WHO [3, 12], and verified via RNA test of SARS-CoV-2 in the specialized lab for clinical research in Nanjing Second Hospital. The vaccination recommendations followed the COVID-19 vaccination technical guidelines of the NHC of China [12]. Two inactivated SARS-CoV-2 vaccines (BBIBP-CorV or CoronaVac) were available in Nanjing city before and during the study period. Two doses of the inactivated vaccines were recommended, with an interval of 3 to 8 weeks [12].

Definitions

The clinical classification of COVID-19 was recommended by the NHC of China [12, 13]: Mild, with minor clinical signs (such as fever and cough) and no imaging manifestations. Moderate, with indications of respiratory tract infections and pneumonia-like imaging characteristics. Severe, having satisfied one or more of the conditions below: (1) respiratory discomfort and a breathing rate of more than 30 breaths per minute; (2) At rest, the pulse oxygen saturation (SpO2) is less than or equal to 93 percent; (3) arterial partial pressure of oxygen (PaO2)/ fraction of inspired oxygen (FiO2) ≤300 mmHg (1 mmHg = 0.133 kPa). Critical, having satisfied one of the criteria below: (1) respiratory failure accompanied by mechanical ventilation (MV); (2) shock; (3) admission into the ICU as a result of multiple organ dysfunction. Sepsis was described as fatal organ failure produced by a dysfunctional host defense against pathogens, whereas septic shock was described as a subtype of sepsis characterized by metabolic/cellular and circulatory impairment that is linked to a greater risk of death [14, 15].

The Berlin standards for acute respiratory distress syndrome (ARDS) were used in making the diagnosis [16]. The presence of liver damage was determined when the serum concentrations of hepatic biological markers (e.g., alanine aminotransferase) exceeded twice the reference upper limit, or when there was an abnormally elevated level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) when especially in comparison with alkaline phosphatase levels [17]. It was determined that the patient had acute kidney injury (AKI) in accordance with the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [18]. An increase in blood levels of biological markers (e.g., troponin I), exceeding twice the reference upper limit, or the discovery of new aberrations in echocardiography and electro-cardiography, was considered evidence of cardiac damage [17]. During the 365-day period before admission to the hospital, comorbidity was considered as present when there was at least 1 specific procedure or 2 specific outpatient procedures, or a prescription for a medicine that characterized the comorbid condition in the 365-day period. Multiple organ dysfunction syndromes (MODS) are recognized as the simultaneous malfunctions of two or more organs that have been identified in an individual.

Data collection

The baseline clinical features, which included body mass index (BMI), age, and sex, days from occurrence to hospitalization, days from vaccination to admission,

days from onset to SARS-CoV-2 testing negative (days of "turn negative"), early signs and symptoms, clinical classifications, and co-morbidities, were obtained. All of the information was derived from electronic medical records, which had to be manually retrieved and the information of each patient was also checked by another investigator. The serum levels of lymphocyte count, white blood cell (WBC) count, ALT, C-reactive protein (CRP), creatinine, D-dimer, brain natriuretic peptide (BNP), procalcitonin (PCT), troponin I (TNI), and interleukin-6 (IL-6) were obtained upon admission. The serum levels of percentages of CD4 and CD8 lymphocytes, virus immunoglobulin (Ig) M and IgG antibody, and the cycle threshold (CT) of RT-PCR assays of admission were also acquired. The professional clinical laboratory of Nanjing Second Hospital was responsible for detecting all of the hematological parameters.

The number of patients with septic shock, cardiac injury, AKI, MODS, liver damage, and ARDS, and patients requiring high-flow nasal cannula (HFNC) or noninvasive ventilation (NIV), continuous renal replacement therapy (CRRT), MV, or extracorporeal membrane oxygenation (ECMO) were collected. The thromboembolic events (e.g., cerebral infarction, cerebral infarction, venous thromboembolism) were also counted. The length of NIV or HFNC, length of hospital stay (LOS), MV-free days, and ICU-free days within the initial 28 days and the 28-day mortality were also collected.

Statistical analysis

The Kolmogorov-Smirnov test was the first to be performed to evaluate the normal distribution of data. Data with normal distributions were presented as the means ± standard deviation with comparisons made using *t*-tests. Data with abnormal distributions were presented as the medians (interquartile ranges, IQR) with comparisons made with the help of the Kruskal-Wallis test and Mann-Whitney *U* test. In this study, categorical data were reported as percentages or absolute numbers, and they were evaluated utilizing the Fisher's exact or χ^2 test. Additionally, we performed an analysis of variance (ANOVA) for multiple testing of the general linear model in order to take into consideration the repetitiveness of the variables. The analysis of statistical data was carried out utilizing IBM Statistical Package for the Social Sciences (SPSS, version 22.0, New York, USA) program, and $P < 0.05$ was established as the criterion of statistical significance. Qiao Liu, a biostatistician from the Jiangsu Provincial Center for Disease Control and Prevention in China, examined the statistical techniques used in this research.

RESULTS

During the course of this clinical retrospective analysis, 181 older individuals with verified COVID-19 (Delta variant) infection were included. The median age was 69 (interquartile range, 65.5–74) years, with 107 (59.1 percent) of the participants being female. Among these patients, 113 (62.4%) were not vaccinated, 46 (25.4%) received one dose of vaccine, and only 22 (12.2%) received two doses of vaccine. One hundred and forty-five (80.1%) patients were categorized as moderate, 21 (11.6%) patients were categorized as severe, and 15 (8.3%) patients were categorized as critical. One hundred and eleven (61.3%) patients had one or more co-morbidities. MODS occurred in 14 patients (7.7% of the total), while septic shock occurred in 12 individuals (6.7% of the total). Two (1.1%) critically ill patients died within 28 days of admission. Table 1 contained the comprehensive clinical information of the patients.

We classified the patients into three groups on the basis of their vaccination status: no vaccination (group A, n = 113), one dose of vaccination (group B, n = 46), and two doses of vaccination (group C, n = 22). As described in Table 2, the days from vaccination to admission in Group B were considerably lower as opposed to those in Group C (P = 0.035). The days of "turn negative" and hospital stay in Group C were substantially reduced as opposed to those in Group A or Group B ($P < 0.05$). The serum levels of TNI, BNP, and PCT in Group C were remarkably reduced as opposed to those in Group A or Group B ($P < 0.05$). The serum TNI and BNP levels in Group B were also reduced in contrast with those in Group A ($P < 0.05$). The levels of virus IgM and IgG antibodies in Group C were considerably elevated as opposed to those in Group A or Group B ($P < 0.05$). The levels of virus IgM and IgG antibodies in Group B were also substantially elevated in contrast with those in Group A ($P < 0.05$).

Table 3 highlighted the differences in clinical severity and outcome characteristics across the 3 groups. The incidences of MODS, septic shock, ARDS, AKI, cardiac injury, and other complications in Group A were remarkably elevated as opposed to the ones in Group B or Group C ($P < 0.05$). The proportions of patients receiving HFNC/NIV or MV in Group A were also considerably increased compared to those in Group B or Group C ($P < 0.05$). However, no differences in the abovementioned parameters were discovered between Group B and Group C ($P > 0.05$). The ICU-free and MV-free days within the initial 28 days in Group A were dramatically reduced in contrast with those in Group B or Group C ($P < 0.05$). No difference was identified in the 28-day mortality among the three

Table 1. Demographic data and clinical parameters (*n* = 181).

Variables	Values
Age (years)	69 (65.5–74)
Sex (Male: Female)	74:107
BMI (kg/m^2)	23.7 (22.2–26.6)
Days from onset to admission	3 (2–5)
Days from vaccination to admission	14 (8–24)
Days of “turn negative”	23 (18–27)
Initial symptoms or signs (*n*, %)	
Fever	60 (33.1%)
Cough	47 (26.0%)
Fatigue	19 (10.5%)
Pharyngalgia	10 (5.5%)
Headache or dizziness	8 (4.4%)
Stuffy nose	6 (3.3%)
Chest tightness or pain	6 (3.3%)
Anorexia	5 (2.8%)
Diarrhea	5 (2.8%)
Nausea or vomiting	4 (2.2%)
Myalgia	3 (1.7%)
Other	8 (4.4%)
Classifications (*n*, %)	
Mild	0 (0%)
Moderate	145 (80.1%)
Severe	21 (11.6%)
Critical	15 (8.3%)
Co-morbidities (repeated)	
Hypertension	82 (45.3%)
Diabetes mellitus	28 (15.5%)
Chronic respiratory diseases	15 (8.3%)
Coronary heart disease	14 (7.7%)
Cerebral infarction	8 (4.4%)
Chronic liver or kidney disease	3 (1.7%)
Other	5 (2.8%)
Blood parameters	
CRP (mg/L)	11.6 (3.4–30.6)
WBC (10^9/L)	4.7 (3.8–6.2)
Lymphocyte (10^9/L)	1.0 (0.8–1.4)
ALT (U/L)	20.5 (15.1–32.1)
Creatinine (umol/L)	64.3 (55.2–78.9)
TNI (pg/mL)	5.6 (1.6–12.4)
D-dimer (mg/L)	0.5 (0.3–0.7)
BNP (pg/mL)	24 (12.2–56.2)
PCT (ng/mL)	0.1 (0.0–0.1)
IL-6 (pg/mL)	24.2 (12.1–37.9)
CD4 T cells percentage (%)	39.0 (33.0–44.5)
CD8 T cells percentage (%)	21 (17–25)
IgM antibody (S/CO)	0.1 (0–0.5)

IgG antibody (S/CO)	0.2 (0.1–1.3)
PCR cycle threshold (CT values)	
ORF1ab gene	23 (20–26)
N gene	20 (17–24)
Organs injury (*n*, %)	
ARDS	15 (8.3%)
Liver injury	11 (6.1%)
AKI	11 (6.1%)
Cardiac injury	12 (6.7%)
MODS	14 (7.7%)
Thrombo-embolic events (*n*, %)	0 (0%)
Septic shock (*n*, %)	12 (6.7%)
Need for NIV/HFNC (*n*, %)	33 (18.2%)
Need for MV (*n*, %)	15 (8.3%)
Need for CRRT/ECMO (*n*, %)	6 (3.3%)
NIV/HFNC days	1.2 ± 3.0
MV-free days	26.4 ± 5.6
ICU-free days	25.4 ± 6.8
Hospital stay (days)	26 (21–30)
Death (*n*, %)	2 (1.1%)

Abbreviations: BMI: body mass index; CRP: C-reactive protein; WBC: white blood cells; ALT: alanine aminotransferase; TNI: troponin I; BNP: brain natriuretic peptide; PCT: procalcitonin; IL-6: interleukin-6; IgM: immunoglobulin M; IgG: immunoglobulin G; PCR: polymerase chain reaction; ARDS: acute respiratory distress syndrome; AKI: acute kidney injury; MODS: multiple organ dysfunction syndrome; NIV: noninvasive ventilation; HFNC: high-flow nasal cannula; MV: mechanical ventilation; CRRT: continuous renal replacement therapy; ECMO: extracorporeal membrane oxygenation; ICU: intensive care unit.

Table 2. The clinical parameters and severity variables.

	Group A (*n* = 113)	**Group B (*n* = 46)**	**Group C (*n* = 22)**	***P* value**
Days from onset to admission	3.0 (2.0–5.0)	4.0 (2.0–5.3)	3.5 (1.8–6.0)	0.701
Days from vaccination to admission	/	12.5 (8.0–20.0)	30.0 (8.0–44.5)	0.035
Days of “turn negative”	23.0 (18.0–27.0)	22.5 (18.0–26.0)	17.0 (13.8–23.3)	0.026
CRP (mg/L)	10.4 (3.4–28.1)	21.1 (4.0–33.9)	6.5 (2.1–26.9)	0.237
WBC (10^9/L)	4.5 (3.5–6.0)	5.0 (4.3–6.2)	5.0 (4.1–6.2)	0.156
Lymphocyte (10^9/L)	1.0 (0.8–1.4)	1.0 (0.7–1.5)	1.1 (0.9–1.5)	0.775
ALT (U/L)	21.2 (15.6–31.1)	19.0 (14.2–32.3)	20.3 (15.1–32.9)	0.638
Creatinine (umol/L)	61.8 (55.3–79.1)	71.8 (55.0–81.7)	60.4 (54.1–73.4)	0.312
TNI (pg/mL)	6.1 (2.2–18.9)	4.1 (1.0–8.3)	3.8 (1.8–9.6)	0.041
D-dimer (mg/L)	0.5 (0.4–0.8)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.507
BNP (pg/mL)	29.2 (12.6–65.3)	20.5 (12.0–44.8)	17.4 (10.0–34.6)	0.039
PCT (ng/mL)	0.06 (0.04–0.1)	0.06 (0.04–0.1)	0.03 (0.02–0.06)	0.043
IL-6 (pg/mL)	25.7 (12.9–39.7)	21.9 (12.6–33.4)	16.3 (8.1–32.7)	0.088
CD4 percentage (%)	38.0 (32.0–43.0)	41.0 (33.8–46.0)	42.0 (37.8–46.3)	0.109
CD8 percentage (%)	21.0 (18.0–25.0)	20.0 (16.8–25.3)	22.0 (16.8–25.5)	0.674
IgM (S/CO)	0.06 (0.03–0.3)	0.2 (0.1–0.8)	0.6 (0.2–2.3)	<0.001

IgG (S/CO)	0.1 (0.06–0.3)	0.3 (0.1–12.5)	10.9 (3.4–104.8)	<0.001
CT values of ORF1ab gene	22.0 (19.0–26.0)	24.0 (20.8–26.0)	22.0 (18.8–25.3)	0.400
CT values of N gene	20.0 (17.0–24.0)	20.0 (17.0–24.0)	21.0 (16.8–24.0)	0.921

Abbreviations: Group A: No vaccination; Group B: One dose of vaccination; Group C: Two doses of vaccination; CRP: C-reactive protein; WBC: white blood cells; ALT: alanine aminotransferase; TNI: troponin I; BNP: brain natriuretic peptide; PCT: procalcitonin; IL-6: interleukin-6; IgM: immunoglobulin M; IgG: immunoglobulin G; CT: cycle threshold.

Table 3. Clinical variables of severity and outcomes.

	Group A (*n* = 113)	Group B (*n* = 46)	Group C (*n* = 22)	*P* value
MODS (*n*, %)	14 (12.4%)	0 (0%)	0 (0%)	0.006
Septic shock (*n*, %)	12 (10.6%)	0 (0%)	0 (0%)	0.011
ARDS (*n*, %)	15 (13.3%)	0 (0%)	0 (0%)	0.004
Liver injury (*n*, %)	8 (7.1%)	2 (4.3%)	1 (4.5%)	0.516
AKI (*n*, %)	10 (8.8%)	1 (2.2%)	0 (0%)	0.048
Cardiac injury (*n*, %)	11 (9.7%)	1 (2.2%)	0 (0%)	0.035
Other complications (*n*, %)	15 (13.3%)	0 (0%)	0 (0%)	0.004
Need for NIV/HFNC (*n*, %)	26 (23.0%)	6 (13.0%)	1 (4.5%)	0.022
Need for MV (*n*, %)	15 (13.3%)	0 (0%)	0 (0%)	0.004
Need for CRRT/ ECMO (*n*, %)	6 (5.3%)	0 (0%)	0 (0%)	0.079
NIV/HFNC days	1.4 ± 3.0	1.2 ± 3.5	0.3 ± 1.5	0.096
MV-free days	25.5 ± 6.9	28 ± 0	28 ± 0	0.008
ICU-free days	24.2 ± 8.2	27.1 ± 2.7	27.6 ± 1.7	0.047
Hospital stay (days)	27 (23–30)	26 (21–28)	20 (17.8–27.3)	0.004
Death (*n*, %)	2 (1.8%)	0 (0%)	0 (0%)	0.315

Abbreviations: Group A: No vaccination; Group B: One dose of vaccination; Group C: Two doses of vaccination; MODS: multiple organ dysfunction syndrome; ARDS: acute respiratory distress syndrome; AKI: acute kidney injury; NIV: noninvasive ventilation; HFNC: high-flow nasal cannula; MV: mechanical ventilation; CRRT: continuous renal replacement therapy; ECMO: extracorporeal membrane oxygenation; ICU: intensive care unit.

groups (P = 0.315). The above findings illustrated that patients with two doses of vaccination may have a lower incidence of organ injury and less requirement for supportive treatments in ICU.

Of the 181 confirmed patients, 111 (61.3%) had comorbidities (e.g., hypertension, diabetes mellitus, and chronic respiratory diseases) before admission. Table 4 demonstrated the differences in the clinical outcome parameters between the vaccinated and no vaccinated patients with or without co-morbidities. In patients with co-morbidities, vaccinated cases had lower incidences of MODS (P = 0.015), septic shock (P = 0.015), and ARDS (P = 0.008). Nevertheless, no significant differences (P > 0.1) were discovered in these prognostic variables between the vaccinated and no vaccinated patients without co-morbidities.

DISCUSSION

This clinical retrospective research examined the roles of inactivated SARS-CoV-2 vaccines in older patients with confirmed infection of Delta variant in Nanjing, China. The vaccination rate of older patients was only 37.6% (68/181). We found that patients with two doses of vaccination may have shorter LOS, ICU stay, and respiratory support time, as well as a lower incidence of organ injury and less requirement for supportive treatments. Moreover, in patients with co-morbidities, vaccinated cases had a lower prevalence of ARDS, septic shock, and MODS. However, no difference was found in 28-day mortality across the different groups.

As a serious global epidemic, the COVID-19 is still not alleviated in lots of countries. Apart from traditional

Table 4. Clinical variables of severity and outcomes in patients with or without co-morbidities.

	Co-morbidities (*n* = 111)		*P* value	No co-morbidities (*n* = 70)		*P* value
	Vaccinated (*n* = 38)	No Vaccinated (*n* = 73)		Vaccinated (*n* = 30)	No Vaccinated (*n* = 40)	
MODS (*n*, %)	0 (0%)	11 (15.1%)	0.015	0 (0%)	3 (7.5%)	0.255
Septic shock (*n*, %)	0 (0%)	10 (13.7%)	0.015	0 (0%)	2 (5.0%)	0.503
ARDS (*n*, %)	0 (0%)	12 (16.4%)	0.008	0 (0%)	3 (7.5%)	0.255
Liver injury (*n*, %)	1 (2.6%)	6 (8.2%)	0.419	2 (6.7%)	2 (5.0%)	1.000
AKI (*n*, %)	1 (2.6%)	9 (12.3%)	0.160	0 (0%)	1 (2.5%)	1.000
Cardiac injury (*n*, %)	1 (2.6%)	8 (11.0%)	0.162	0 (0%)	3 (7.5%)	0.255
Thrombo-embolic events (*n*, %)	0 (0%)	0 (0%)	/	0 (0%)	0 (0%)	/
Death (*n*, %)	0 (0%)	2 (2.7%)	0.546	0 (0%)	0 (0%)	/

Abbreviations: MODS: multiple organ dysfunction syndrome; ARDS: acute respiratory distress syndrome; AKI: acute kidney injury.

isolation and symptomatic treatments, increasing SARS-CoV-2 vaccines were developed to prevent COVID-19. Thompson et al. [19] reported high efficacy of the two-dose messenger RNA (mRNA) vaccines BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) for the SARS-CoV-2 infection prevention among adults within the working age. Zhang Y and colleagues [20] investigated the safety, tolerance, and ability to induce an immune response of an inactivated CoronaVac vaccine (Sinovac Life Sciences, Beijing, China) in a phase 1/2 clinical trial in China, and they discovered that two dosages of CoronaVac at varied concentrations and dosage regimens were well tolerable and mildly immunogenic among healthy individuals within the age range of 18–59 years old. Jara's study [7] also suggests that the CoronaVac vaccination was successful in preventing COVID-19, which resulted in severe sickness and death in Chile. Unfortunately, the efficacy of the vaccines was still questioned by the emerging mutant variants of SARS-CoV-2.

More and more variants have been reported across the globe: Alpha, Beta, Gamma, Delta, Omicron, and so on [6, 21]. In particular, the Delta variant is considered to be a crucial reason for the recurrence or deterioration of the COVID-19 epidemic [5, 6, 22]. After perfectly controlling the epidemic in 2020, China is also suffering from the sporadic outbreak of the COVID-19 (Delta) in 2021. Consequently, it is necessary to investigate the SARS-CoV-2 vaccine efficacy in Delta variants. Lopez and colleagues [4] found that the ChAdOx1 nCoV-19 and BNT162b2 vaccines targeting the Delta variant were effective after the receipt of two vaccine doses. During the Delta strain epidemic in May 2021 in Guangzhou city, China, Li XN and colleagues [23] confirmed the efficacy of two doses of inactivated vaccines in preventing the Delta variant infection in patients (between the ages of 18–59 years). However, the effectiveness of these vaccines in older patients was less investigated.

Wu Z and colleagues confirmed that the CoronaVac was safe and well-tolerated in older adults [10]. Another study also confirmed that the CoronaVac vaccination was successful in the prevention of COVID-19 as well as the associated severe illness and death in older adults [7]. On July 21, 2021, an imported COVID-19 epidemic attributed to the Delta strain was reported in Nanjing city of China [3]. When undertaking our clinical efforts to control the COVID-19 pandemic (Delta variant) in Nanjing, we discovered that there were a number of older and vaccinated patients among confirmed cases. In addition, 61.3% of the older patients had one or more co-morbidities. Therefore, we investigated the roles of Chinese inactivated SARS-CoV-2 vaccines (BBIBP-CorV or CoronaVac) in older patients with confirmed infection of Delta variant, especially in those with co-morbidities in this study. Our results suggested that two doses of the vaccines were effective in improving the disease severity of older patients (aged ≥60 years) with Delta variant, including those with co-morbidities. No difference in 28-day mortality was observed, which might be attributed to the limited sample size employed in this retrospective study.

The immune status and viral load (CT value) of older patients were also investigated in this study. T cell receptor diversity may decline with age in both CD8 and CD4 cells, reducing T cell survival [11]. A study by Thompson MG found that vaccination reduced the load of viral RNA present, the likelihood of febrile manifestations, and the disease duration for those who experienced breakthrough infections despite having received vaccination [19]. The findings of our research

demonstrated the differences in immunoglobulin (IgM, IgG) levels in patients with different doses of the vaccines. However, no differences were found in the CD4 or CD8 percentages and the CT values of RT-PCR assays. These results were inconsistent with previous reports, which might attribute to the differences in the days from vaccination to admission [12.5 (8–20) VS. 30 (8–44.5), $P = 0.035$] in our study.

The study had some limitations. It is possible that the results are inconclusive because of the limited sample size and single-center retrospective methodology; hence, large-scale clinical prospective research needs to be carried out to determine the correctness of these findings. Since this research did not employ patho-physiology models and the findings were hypothesis-generating, it is necessary to do more fundamental tests in order to determine the actual processes of vaccinations in older individuals who had suffered from infection with the Delta variant. Finally, because some variables were only collected on admission, the later effects of vaccines on these variables need to be examined in the following clinical studies.

In summary, this clinical retrospective investigation confirmed that the inactivated SARS-CoV-2 vaccines were efficacious in improving the disease severity of older patients with infection of the Delta variant, especially in those with co-morbidities.

AUTHOR CONTRIBUTIONS

Sun JK, Wang X, and Shi QK designed the research; Song XC, Sun JK, Zhang WH, Shen X, Xu H, Nie S, Xiao JL, Sun F, Shu C, Chen JD, Tang Y, and Feng P performed the research; Song XC, Zhou XH, Cheng JH, Wang X, and Sun XP analyzed the data; Song XC, Zhou XH, Sun JK, and Sun XP wrote the paper.

ACKNOWLEDGMENTS

The authors thank Qiao Liu for her assistance in the statistical analysis of this study. The authors also thank Wang Y, Qi JW, Gao L, Chen YB, Yuan Y, Lei J, and other colleagues of Jiangsu united medical team.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to this study.

Editorial note

&This corresponding author has a verified history of publications using a personal email address for correspondence.

REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, et al, and China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020; 382:727–33. https://doi.org/10.1056/NEJMoa2001017 PMID:31978945

2. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. JAMA. 2020; 324:782–93. https://doi.org/10.1001/jama.2020.12839 PMID:32648899

3. World Health Organization. Coronavirus disease (COVID-19). https://www.who.int.

4. Lopez Bernal J, Andrews N, Gower C, Gallagher E, Simmons R, Thelwall S, Stowe J, Tessier E, Groves N, Dabrera G, Myers R, Campbell CNJ, Amirthalingam G, et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. N Engl J Med. 2021; 385:585–94. https://doi.org/10.1056/NEJMoa2108891 PMID:34289274

5. Weber S, Ramirez CM, Weiser B, Burger H, Doerfler W. SARS-CoV-2 worldwide replication drives rapid rise and selection of mutations across the viral genome: a time-course study - potential challenge for vaccines and therapies. EMBO Mol Med. 2021; 13:e14062. https://doi.org/10.15252/emmm.202114062 PMID:33931941

6. Wang R, Zhang Q, Ge J, Ren W, Zhang R, Lan J, Ju B, Su B, Yu F, Chen P, Liao H, Feng Y, Li X, et al. Analysis of SARS-CoV-2 variant mutations reveals neutralization escape mechanisms and the ability to use ACE2 receptors from additional species. Immunity. 2021; 54:1611–21.e5. https://doi.org/10.1016/j.immuni.2021.06.003 PMID:34166623

7. Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, Pizarro A, Acevedo J, Leo K, Leon F, Sans C, Leighton P, Suárez P, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. N Engl J Med. 2021; 385:875–84. https://doi.org/10.1056/NEJMoa2107715 PMID:34233097

8. Yang S, Li Y, Dai L, Wang J, He P, Li C, Fang X, Wang C, Zhao X, Huang E, Wu C, Zhong Z, Wang F, et al. Safety and immunogenicity of a recombinant tandem-repeat dimeric RBD-based protein subunit vaccine (ZF2001)

against COVID-19 in adults: two randomised, double-blind, placebo-controlled, phase 1 and 2 trials. Lancet Infect Dis. 2021; 21:1107–19. https://doi.org/10.1016/S1473-3099(21)00127-4 PMID:33773111

9. Al Kaabi N, Zhang Y, Xia S, Yang Y, Al Qahtani MM, Abdulrazzaq N, Al Nusair M, Hassany M, Jawad JS, Abdalla J, Hussein SE, Al Mazrouei SK, Al Karam M, et al. Effect of 2 Inactivated SARS-CoV-2 Vaccines on Symptomatic COVID-19 Infection in Adults: A Randomized Clinical Trial. JAMA. 2021; 326:35–45. https://doi.org/10.1001/jama.2021.8565 PMID:34037666

10. Wu Z, Hu Y, Xu M, Chen Z, Yang W, Jiang Z, Li M, Jin H, Cui G, Chen P, Wang L, Zhao G, Ding Y, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021; 21:803–12. https://doi.org/10.1016/S1473-3099(20)30987-7 PMID:33548194

11. Soiza RL, Scicluna C, Thomson EC. Efficacy and safety of COVID-19 vaccines in older people. Age Ageing. 2021; 50:279–83. https://doi.org/10.1093/ageing/afaa274 PMID:33320183

12. National Health Commission of the People's Republic of China. http://www.nhc.gov.cn.

13. Sun JK, Liu Y, Zou L, Zhang WH, Li JJ, Wang Y, Kan XH, Chen JD, Shi QK, Yuan ST. Acute gastrointestinal injury in critically ill patients with COVID-19 in Wuhan, China. World J Gastroenterol. 2020; 26:6087–97. https://doi.org/10.3748/wjg.v26.i39.6087 PMID:33132657

14. Levy MM, Evans LE, Rhodes A. The Surviving Sepsis Campaign Bundle: 2018 Update. Crit Care Med. 2018; 46:997–1000. https://doi.org/10.1097/CCM.0000000000003119 PMID:29767636

15. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017; 43:304–77. https://doi.org/10.1007/s00134-017-4683-6 PMID:28101605

16. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS, and ARDS Definition Task Force. Acute respiratory distress syndrome: the Berlin Definition. JAMA. 2012; 307:2526–33. https://doi.org/10.1001/jama.2012.5669 PMID:22797452

17. Sun JK, Zhang WH, Zou L, Liu Y, Li JJ, Kan XH, Dai L, Shi QK, Yuan ST, Yu WK, Xu HY, Gu W, Qi JW. Serum calcium as a biomarker of clinical severity and prognosis in patients with coronavirus disease 2019. Aging (Albany NY). 2020; 12:11287–95. https://doi.org/10.18632/aging.103526 PMID:32589164

18. Section 2: AKI Definition. Kidney Int Suppl (2011). 2012; 2:19–36. https://doi.org/10.1038/kisup.2011.32 PMID:25018918

19. Thompson MG, Burgess JL, Naleway AL, Tyner H, Yoon SK, Meece J, Olsho LEW, Caban-Martinez AJ, Fowlkes AL, Lutrick K, Groom HC, Dunnigan K, Odean MJ, et al. Prevention and Attenuation of Covid-19 with the BNT162b2 and mRNA-1273 Vaccines. N Engl J Med. 2021; 385:320–9. https://doi.org/10.1056/NEJMoa2107058 PMID:34192428

20. Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, Han W, Chen Z, Tang R, Yin W, Chen X, Hu Y, Liu X, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021; 21:181–92. https://doi.org/10.1016/S1473-3099(20)30843-4 PMID:33217362

21. Tao K, Tzou PL, Nouhin J, Gupta RK, de Oliveira T, Kosakovsky Pond SL, Fera D, Shafer RW. The biological and clinical significance of emerging SARS-CoV-2 variants. Nat Rev Genet. 2021; 22:757–73. https://doi.org/10.1038/s41576-021-00408-x PMID:34535792

22. Salian VS, Wright JA, Vedell PT, Nair S, Li C, Kandimalla M, Tang X, Carmona Porquera EM, Kalari KR, Kandimalla KK. COVID-19 Transmission, Current Treatment, and Future Therapeutic Strategies. Mol Pharm. 2021; 18:754–71. https://doi.org/10.1021/acs.molpharmaceut.0c00608 PMID:33464914

23. Li XN, Huang Y, Wang W, Jing QL, Zhang CH, Qin PZ, Guan WJ, Gan L, Li YL, Liu WH, Dong H, Miao YT, Fan SJ, et al. Effectiveness of inactivated SARS-CoV-2 vaccines against the Delta variant infection in Guangzhou: a test-negative case-control real-world study. Emerg Microbes Infect. 2021; 10:1751–9. https://doi.org/10.1080/22221751.2021.1969291 PMID:34396940

5.5. CoronaVac induz resposta imune em mais de 80% dos idosos, mostra estudo turco

Um estudo feito na Turquia com idosos imunizados com CoronaVac voltou a mostrar que a vacina confere alta proteção nesse público, induzindo produção de anticorpos em 80,9% dos indivíduos. O artigo foi publicado na edição de maio da revista Age & Aging e conduzido por pesquisadores da Faculdade de Medicina da Universidade de Ancara, capital turca.

Os cientistas avaliaram 497 pacientes idosos com idade média de 72 anos que foram vacinados com CoronaVac. A taxa de soroconversão (indivíduos que produziram anticorpos) após um a dois meses da segunda dose foi de 80,9%, se mantendo alta até o terceiro mês, com 73,2%.

O estudo também apontou que a presença de comorbidades, como doenças crônicas, pode influenciar na resposta imune, ainda mais em indivíduos mais velhos que já têm o sistema imunológico comprometido naturalmente devido ao envelhecimento. Por isso, é muito importante que essa população receba a dose de reforço. A terceira dose da CoronaVac já se mostrou eficaz para potencializar a imunidade dos idosos, aumentando em quase dez vezes a capacidade neutralizante contra o SARS-CoV-2.

Evidências científicas

Diversos estudos feitos com dados de mundo real já comprovaram que a vacina do Butantan e da Sinovac é segura e protege idosos contra casos graves e mortes. No Projeto S, por exemplo, estudo de efetividade da CoronaVac conduzido em Serrana, interior de São Paulo, o imunizante administrado em idosos protegeu 86,4% contra casos sintomáticos, 96,9% contra hospitalizações e 96,9% contra mortes.

Outro estudo brasileiro feito com 60 milhões de pessoas, baseado nos dados nacionais do Sistema de Vigilância Epidemiológica da Gripe (SIVEP-Gripe) do Ministério da Saúde, apontou uma proteção da CoronaVac de 84,2% contra hospitalizações, 80,8% contra internações em UTI e 76,5% contra mortes.

Publicado em: 6/5/2022

Age and Ageing 2022; **51:** 1–9
https://doi.org/10.1093/ageing/afac088

RESEARCH PAPER

Antibody response with SARS-CoV-2 inactivated vaccine (CoronaVac) in Turkish geriatric population

Arzu Okyar Baş[1], Merve Hafizoğlu[1], Filiz Akbiyik[2], Merve Güner Oytun[1], Zeynep Şahiner[1], Serdar Ceylan[1], Pelin Ünsal[1], Burcu Balam Doğu[1], Mustafa Cankurtaran[1], Banu Çakir[3], Serhat Ünal[4], Meltem Gülhan Halil[1]

[1]Department of Internal Medicine, Division of Geriatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey
[2]Ankara City Hospital Laboratory, Siemens Healthineers, Ankara, Turkey
[3]Department of Public Health, Hacettepe University Faculty of Medicine, Ankara 06230, Turkey
[4]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Address correspondence to: Arzu Okyar Baş. Tel: +905065630765; Fax: +903123052302. Email: arzu0506@hotmail.com

Abstract

Background: Sars-CoV-2 infection influences older individuals at the forefront, and there is still limited data on the COVID-19 vaccine response in the geriatric population. This study aimed to assess antibody response after vaccination with SARS-CoV-2 inactivated vaccine and examine possible factors affecting this response in a geriatric population.
Methods: individuals who have been on at least the 28th day after the second dose of the COVID-19 vaccine were included. Comprehensive geriatric assessment tools and the Clinical Frailty Scale were performed. SARS-CoV-2 spike-specific IgG antibodies were detected and, levels ≥1 U/ml were defined as seropositive, <1 U/ml were defined as seronegative.
Results: a total of 497 patients were included and divided into three groups according to the days past after the second dose of the vaccine (Group 1: 28–59 days, Group 2: 60–89 days and Group 3: 90 days and more). Groups included 188, 148 and 171 patients, respectively. Seropositivity rate in each group was 80.9,73.2 and 57.3%, respectively. In Groups 1 and 2, Charlson Comorbidity Index score was higher in the seronegative group ($P = 0.023$ and $P = 0.011$, respectively). In Group 3, the prevalence of frailty was significantly higher in the seronegative group ($P = 0.002$).
Conclusion: to the best of our knowledge, this is the first study assessing the antibody response after vaccination with Sars-CoV 2 inactivated vaccine in the Turkish geriatric population. Moreover, this is the first study revealing the relationship between antibody response and frailty. Larger studies are needed to confirm the antibody response duration and the association between frailty and COVID-19 vaccine response.

Keywords: SARS-CoV-2, sinovac, coronavac, neutralising antibody, anti-Spike IgG, older people

Key Points

- Seroconversion rate in older adults significantly decreased 90 days after the second dose of vaccine.
- Frailty might play an important role in vaccine response.
- The seropositivity rate was significantly lower in frail geriatric patients after two-dose scheduled inactive SARS-CoV-2 vaccination.

Introduction

Sars-CoV2 infection undoubtedly influenced older individuals at the forefront. Although important steps have been taken worldwide for the treatment and prevention, vaccination is the most powerful weapon to break the chain of transmission.

Sinovac's Coronavac vaccine is an inactivated whole virus vaccine approved by 32 countries, including Turkey, for use in adults ≥ 18 years. [1] Despite the disadvantages (i.e. the integrity of antigens or epitopes that should be verified, limited immunogenicity requiring adjuvants to enhance the immune response), inactivated vaccines are still popular due to their advantages (i.e. non-replicability in the host, non-transmissibility, relatively easy production systems) [2]. Most vaccine studies (prepared by either new or conventional methods; mRNA, adenovirus vector, adjuvant protein or inactivated virus), have not included older patients, especially the frail groups. Therefore, the immune response to vaccines in this special group is not well known [2, 3].

Vaccine response in older adults is not a truly well-understood area [4]. Immunosenescence (qualitative and quantitative deterioration in immune response due to ageing), frailty and multiple chronic diseases make it difficult to predict the vaccine response in the geriatric population. Furthermore, there is insufficient data on the duration of the antibody response. A study conducted on inactivated influenza vaccine reported that seroprotection rates against all three strains in the vaccine had decreased six months after vaccination in older individuals [5]. Therefore, it is essential to highlight the duration of seroprotection after vaccination with inactivated SARS-CoV-2 vaccine in this population.

The aim of this study was to assess antibody response after vaccination with SARS-CoV-2 inactivated vaccine (CoronaVac) and to examine possible factors that may affect this response in a geriatric population aged 60 years and older, who were evaluated in terms of frailty with comprehensive geriatric assessment (CGA).

Materials and methods

Sample size determination

In a study with a dichotomous (yes/no) endpoint (Sars-CoV2 infection) and a study group (older adults) from the community, it was predicted that the risk of COVID-19 infection in the known (older adults) population was 2% and the risk of infection in this population could be reduced by 85% with inactivated vaccine. Thus, 411 people aged 60 or over should be included in the study group with a margin of error of 0.05 (alpha) and power of 90% [6]. Assuming there may be a 20% loss until the end of the study, it was calculated that the sample size should be 493 with 20% excess.

Study design

Four hundred ninety-seven geriatric outpatients, who were 60 years and older and who were on at least the 28th day after the second dose of the SARS-CoV-2 inactivated vaccine (CoronaVac), and had not met the exclusion criteria were enrolled for the study. Exclusion criteria were determined as any history of Sars-CoV-2 real-time PCR or thorax computer tomography proven or clinically suspected COVID-19 infection (information confirmed from the national database), immunosuppressive treatments, patients with dementia, active oncological treatments and regular dialysis treatment.

Demographic data of the participants (age, gender, education, occupation, where and whom they live with), chronic diseases, medications, polypharmacy, smoking and falls were recorded.

Comprehensive geriatric assessment

CGA was performed using standardised tools, i.e. Mini-Mental State Examination (MMSE), Mini Nutritional Assessment short-form (MNA-SF), Yesavage's Geriatric Depression Scale (YGDS), The Katz Activities of Daily Living (ADL) scale and Lawton-Brody Instrumental Activities of Daily Living (IADL). The patient's functional status was evaluated using the Katz ADL test, evaluating over 6 points by questioning how independently the patient performed basic care and activities related to daily life and the score increased as independence increased [7]. The Lawton Brody scale was performed to evaluate patients' IADLs [8]. The cognitive status of the participants was screened by MMSE. The patients' orientation, memory, attention, calculation, recall, language, motor function and perception skills were assessed with the MMSE. The maximum score of the test is 30 points, and the scores 24 and below were assessed as cognitive impairment [9]. Nutritional screening via MNA-SF was performed and, scores > 11 points were defined as normal, 8–11 points were defined as the risk of malnutrition and ≤7 points were defined as malnutrition [10]. The YGDS was used for depression screening, and patients scoring over five points were assessed clinically for depression [11].

Assessment of frailty

The Clinical Frailty Scale (CFS) was performed to assess frailty. CFS defines clinical frailty by giving a score between 1 and 9 (1: very fit; 2: well; 3: well with the treated comorbid disease; 4: apparently vulnerable; 5: mildly frail; 6: moderately frail; 7: severely frail; 8: very severely frail; and 9: terminally ill) based on the clinical opinion of the physician, and according to accepted definitions, patients were divided into two groups as non-frail (CFS ≤ 4) and frail (CFS > 4) [12]. Turkish validation study of CFS was available, and CFS was found to be a reliable and valid frailty screening tool for community-dwelling older adults in the Turkish population [13].

Muscle strength

Muscle strength was evaluated by handgrip measurements defined via the Takei grip strength dynamometer. The

Table 1. Demographical characteristics and Comprehensive Geriatric Assessments according to groups defined as the days past after the second dose of the vaccine

		Group 1 (28–59 days past after the second dose of vaccination group) ($n = 188$ (37.8%)	Group 2 (60–89 days past after the second dose of vaccination group) $n = 148$ (27.8%)	Group 3 (90 and more days past after the second dose of vaccination group) $n = 171$ (34.4%)	*P* value
Age, median (IQR)		71 (67–75)	71 (67–73)	75(67–79)	<0.0001[a,c,d]
Female gender, *n* (%)		111 (56.%)	91 (65.9%)	103 (60.2%)	0.419
Comorbidities	Depression, *n* (%)	18 (9.6%)	19 (13.8%)	24 (14%)	0.406
	CVD, *n* (%)	49 (26.1%)	30 (21.7%)	48 (28.1%)	0.438
	HT, *n* (%)	135 (71.8%)	101 (73.2%)	122 (71.3%)	0.934
	DM, *n* (%)	84 (44.7%)	53 (38.4%)	67 (39.2%)	0.434
	Atrial fibrillation, *n* (%)	16 (8.5%)	11 (8.0%)	25 (14.6%)	0.089
	Hypothyroidism, *n* (%)	34 (18.1%)	28 (20.3%)	28 (16.4%)	0.674
	Congestive heart failure, *n* (%)	19 (10.1%)	10 (7.2%)	14 (8.2%)	0.643
	Rheumatological diseases, *n* (%)	20 (10.6%)	9 (6.5%)	15 (8.8%)	0.433
	Malignancy history, *n* (%)	25 (13.3%)	14 (10.1%)	17 (9.9%)	0.535
	Chronic renal disease, *n* (%)	8 (4.3%)	9 (6.5%)	8 (4.7%)	0.630
	Pulmonary diseases, *n* (%)	15 (8.0%)	13 (9.4%)	6 (3.4%)	0.091
Basic ADLs, median (IQR)		6 (6–6)	6 (5–6)	6 (5–6)	0.051
Instrumental ADLs, median (IQR)		8 (8–8)	8 (8–8)	8 (7–8)	0.143
MMSE, median (IQR)		28 (26–30)	28 (25–29)	28 (26–30)	0.177
Geriatric Depression Scale score, median (IQR)		1(0–5)	2 (0–4.5)	2 (0–4)	0.506
Clock drawing test, median (IQR)		5 (3–6)	6 (3–6)	6 (3–6)	0.669
CCI Score, median (IQR)		1 (0–2)	1 (0–2)	1 (0–2)	0.060
CFS score, median (IQR)		3 (3–4)	3 (3–4)	3 (3–4)	0.071
CFS-frailty, *n* (%)		37 (20.2%)	28 (21.2%)	36 (22%)	0.924
MNA-SF score, median (IQR)		14 (12–14)	14 (12–14)	14 (12–14)	0.138
Malnutrition (MNA < 12)		43 (23.9%)	20 (15.9%)	41 (27.5)	0.066
Number of drugs, median (IQR)		5 (3–7)	5 (3–7)	5 (2–7)	0.744
Polypharmacy, *n* (%)		110 (59.5%)	75 (54.7%)	100 (58.5%)	0.520
Falls, *n* (%)		26 (14.6%)	22 (17.7%)	23 (16%)	0.764
HGS, median (IQR)		20 (16–29.3)	20 (17–26.6)	21.2 (17.2–29.7)	0.755
Low HGS, *n* (%)		60 (38.7%)	32 (28.8%)	47 (36.7%)	0.228
Low gait speed, *n* (%)		49 (33.8%)	27 (26.5%)	37 (30.1%)	0.465
Gait speed, median (IQR)		0.9 (0.7–1.2)	0.9 (0.7–1.0)	0.9 (0.8–1.1)	0.881
Sars-CoV2 spike IgG serum level U/ml, median (IQR)		4 (1.6–10)	1.78 (0.9–5.3)	1.35 (0.5–3.7)	<0.0001[a,b,c,d]
Sars-CoV2 spike IgG serum level BAU/ml, median (IQR)		79.1 (31.6–166.0)	41.2 (19.8–103.5)	27.0 (10.9–62.3)	<0.0001[a,b,c,d]
Seropositivity, *n* (%)		152 (80.9%)	101 (73.2%)	98 (57.3%)	<0.0001[a,c,d]

CVD, Cardiovascular diseases; HT, Hypertension; DM, Diabetes Mellitus; ADL, Activities of daily living; MMSE, Mini-mental State Examination; CFS, Clinical Frailty Scale; MNA-SF, Mini Nutritional Assessment short-form. [a]*P*-value < 0.05 for the comparison between Group 1, 2 and 3. [b]*P* value < 0.05 for the comparison between Group 1 and 2. [c]*P* value < 0.05 for the comparison between Group 1 and 3. [d]*P* value < 0.05 for the comparison between Group 2 and 3.

measurements were made three times with the dominant hand in the sitting position, with the elbow bent at 90° and the hand in the neutral position. The highest of the three repeated measurements was used in the analysis. Cut-off values were taken according to the EWSGOP revised sarcopenia criteria, the low handgrip strength (HGS) for women and men, was described as HGS < 16 kg and <27 kg, respectively [14].

Physical performance

The gait speed measurement was utilised to assess physical performance. In the four-metres walking test, the patient was asked to walk at a normal speed (with the auxiliary device if used) and stop at a specified point, and the elapsed time was recorded in seconds, then the patient's walking speed was calculated in m/s. Values below 0.8 m/s were evaluated in favour of low physical performance [14].

Assessment of comorbidities

We used the Charlson comorbidity index (CCI) to assess the patients' comorbidities. CCI is a commonly used comorbidity index including 17 comorbidities, and it indicates disease burden with robust estimation of mortality [15, 16].

Detection of SARS-CoV-2 IgG antibody

To measure the level of IgG against SARS-CoV-2, blood samples were drawn from the patients. Serum samples were collected by whole blood centrifugation at 4,000 rpm for 10 min. All samples were stored at –20°C before testing. Atellica IM SARS-CoV-2 IgG (sCOVG) assay (11207386, California, USA) was used to detect IgG against

the SARS-CoV-2 spike protein receptor-binding domain and all samples were run in Atellica IM 1600 analyser (Siemens Healthineers, California, USA). The Atellica IM sCOVG assay is a fully automated two-step sandwich immunoassay using chemiluminescent technology with a measuring interval between 0.50–150.00 Index (U/ml). The result is reported as non-reactive (negative) if the value is <1.00 U/ml, and as reactive (positive) if the value is ≥1.00 U/ml. The analytical sensitivity at the cut-off values for the Atellica IM sCOVG assay was determined using the World Health Organization First International Standard for anti-SARS-CoV-2 immunoglobulin (human) NIBSC code: 20/136. The concentration of the reference standard that corresponds to the cut-off value of 1.00 Index (U/ml) for the assay is 21.80 BAU/ml [17].

Statistical analyses

Statistical analyses were executed by SPSS version 22.0 (IBM). Variables were investigated using visual (histogram, probability plots) and analytic methods to determine whether or not they are normally distributed. Descriptive statistics were presented as mean ± standard deviation for variables with normal distribution, median (IQR) for disproportionate variables and the number of cases and (%) for nominal variables. In terms of median values, when the group number was two, the differences between the groups were investigated by Mann–Whitney U test. When the group number was more than two, the differences between the groups were investigated by the Kruskal–Wallis test. Chi-square test or Fisher exact test were performed for categorical variables to compare the data and Bonferroni correction was performed when necessary. A P value < 0.05 was considered statistically significant.

Results

A total of 497 patients with a median (IQR) age of 72 (67–78) years were enrolled in the study, and 305 (61.4%) patients were female. The median (IQR) number of days after the 2nd dose of vaccine was 72 (45–97) days. The seropositivity rate in the whole sample was 70.6% ($n = 351$).

In order to evaluate the course of the antibody response over time, patients were divided into three groups according to the days past after the second dose of the vaccine (Group 1: 28–59 days, Group 2: 60–89 days and Group 3: 90 days and more), and groups included 188, 148 and 171 patients, respectively.

Patients in Group 3 were significantly older than other groups ($P < 0.0001$). There were no differences between groups in terms of gender, comorbidities, CGAs, frailty and functional status (Table 1).

Seropositivity rate decreased over time, and the rate in each group was 80.9, 73.2 and 57.3%, respectively (Appendix 1). sCOVG serum level median (IQR) binding

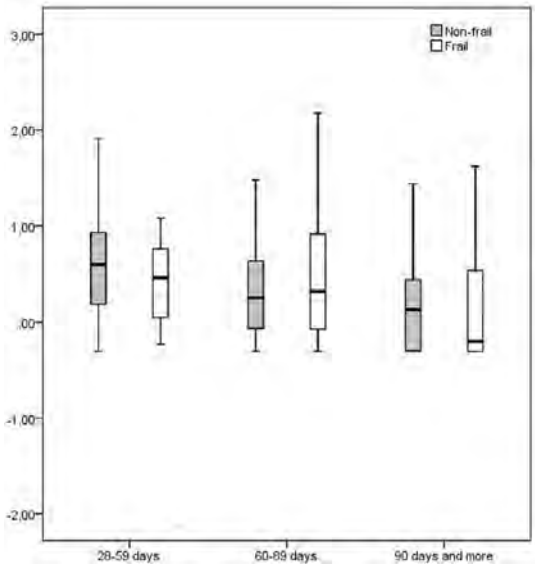


Figure 1. Distribution of antibody titres according to frailty status.

antibody unit (BAU/ml) for each group was 79.1 (31.6–166.0), 41.2 (19.8–103.5) and 27.0 (10.9–62.3), respectively ($P < 0.0001$). In any of the three groups, no significant difference was found between antibody positive and negative groups in terms of age, gender, most comorbidities (exceptions showed in Tables 2 and 3), nutritional status, number of drugs, falls, low HGS, low gait speed, presence of adverse reactions and the scores of Basic ADLs, MMSE, YGDS and clock drowning test (Tables 2–4). Distribution of seropositivity according to the days past after the second dose of the vaccine is given in Supplementary Appendices 1. In Groups 1 and 2, the CCI scores were higher in the seronegative group ($P = 0.023$ and $P = 0.011$, respectively) (Tables 2 and 3). In Group 3, the median (IQR) of IADL, for seropositive and negative groups were 8 (8–8) and 8 (6–8), respectively ($P = 0.025$), and prevalence of frailty was significantly higher in the seronegative group (13.4 vs. 34.3% for seropositive and negative groups, respectively ($P = 0.002$), Table 4). In Figure 1, solely for using in the box blot plot, the logarithmic spike IgG levels were calculated to exclude outliers. These logarithmic spike IgG levels were only used in Figure 1 to show the distribution of seropositivity rate according to the frailty status more comprehensible.

Discussion

Sars-CoV2 infection is a universal challenge and although Sars-CoV2 infection undoubtedly influenced older individuals at the forefront, there is still limited data on the COVID-19 vaccine response in the geriatric population. However, great strides have been made in vaccination since

Table 2. Comparison of seropositive and seronegative individuals in Group 1

		Sars-CoV2 spike IgG antibody positive group $n = 152$ (80.9%)	Sars-CoV2 spike IgG antibody negative group $n = 36$ (19.1%)	*P* value
Age, median (IQR)		71 (67–76)	72 (68–80.75)	0.272
Female gender *n* (%)		87 (58%)	22 (61.1%)	0.734
Comorbidities	Depression, *n* (%)	16 (10.2%)	2 (5.6%)	0.533
	CVD, *n* (%)	40 (26.7%)	9 (25.0%)	0.838
	HT, *n* (%)	107 (71.3%)	28 (77.8%)	0.436
	DM, *n* (%)	65 (43.3%)	19 (52.8%)	0.307
	Atrial fibrillation, *n* (%)	13 (8.7%)	3 (8.3%)	0.949
	Hypothyroidism, *n* (%)	27 (18.0%)	7 (19.4%)	0.840
	Congestive heart failure, *n* (%)	13 (8.7%)	6 (16.7%)	0.215
	Rheumatological diseases, *n* (%)	13 (8.7%)	7(19.4%)	0.074
	Malignancy history, *n* (%)	18 (12.0%)	7 (19.4%)	0.276
	Chronic renal disease, *n* (%)	6 (4.0%)	2 (5.6%)	0.653
	Pulmonary diseases, *n* (%)	12 (8.0%)	9 (25.0%)	0.008*,**
Basic ADLs, median (IQR)		6 (5.75–6.0)	6 (5.0–6.0)	0.991
Instrumental ADLs, median (IQR)		8 (8–8)	8 (8–8)	0.991
MMSE, median (IQR)		28 (26–30)	27(26–29)	0.377
Geriatric depression scale score, median (IQR)		2(0–5)	1(0–3)	0.204
Clock drawing test, median (IQR)		5 (5–6)	6 (5–6)	0.864
CCI score, median (IQR)		1 (0–2)	2 (1–3)	0.023*
CFS score, median (IQR)		3 (3–4)	3 (3–4)	0.406
CFS-frailty, *n* (%)		29 (19.7%)	8 (22.2%)	0.738
MNA-SF score, median (IQR)		14 (12.7–14)	14 (11–14)	0.968
Malnutrition (MNA < 12)		32 (22.2%)	11 (30.6%)	0.294
Number of drugs, median (IQR)		5 (3–7)	6 (3–8)	0.200
Polypharmacy, *n* (%)		88 (59.9%)	22 (61.1%)	0.891
Falls, *n* (%)		19 (13.2%)	7 (20.6%)	0.285
HGS, median (IQR)		20.0 (16–29.3)	19.9 (16.2–29.4)	0.392
Low HGS, *n* (%)		45 (35.7%)	15 (51.7%)	0.111
Low gait speed, *n* (%)		40 (33.6%)	9 (34.6%)	0.922
Gait speed, median (IQR)		0.90 (0.6–1.2)	0.961 (0.7–1.2)	0.826
Sars-CoV2 spike IgG serum level U/ml, median (IQR)		4.6 (2.5–10)	0.56 (0–0.6)	<0.0001*,**,***
Sars-CoV2 spike IgG serum level BAU/ml, median (IQR)		100.3 (55.1–217.3)	12.3 (0.0–14.2)	<0.0001*,**,***

CVD, Cardiovascular diseases; HT, Hypertension; DM, Diabetes Mellitus; ADL, Activities of daily living; MMSE, Mini-mental State Examination; CFS, Clinical Frailty Scale; MNA-SF, Mini Nutritional Assessment short-form. *Significance at $P < 0.05$. **Significance at $P < 0.01$. ***Significance at $P < 0.001$.

the beginning of the pandemic; older adults, who are likely to be among the first to be vaccinated, are often excluded in vaccine studies. Therefore, evaluating the vaccine response in older adults, who are mostly affected by the pandemic and may have many confounding factors like frailty and multiple chronic comorbidities, is essential. In the light of the CGA, including frailty assessment, this study aimed to evaluate the antibody response and factors that may affect it in this particular group. Our findings suggest that antibody response after two doses of the inactivated vaccine decreases, especially after 90 days. Furthermore, to the best of our knowledge, this study is the first revealing comorbidity burden and frailty as important factors for COVID-19 vaccine seroconversion in the geriatric population.

There is still insufficient data for long-term follow-up of antibody response after COVID-19 vaccination not only in older adults but also in all age groups. A recent study on non-immunocompromised healthcare workers showed a significant decline in neutralising antibody titres three months after the second dose of BNT162b2 [18]. In addition, Sinopharm's inactivated COVID-19 vaccine, which has similar technology with Sinovac's Coronavac, also showed decreased antibody production, vaccine effectiveness and mortality reduction, in older adults [19, 20]. Similar to the previous studies, we observed a significant decline in the seroconversion rate over time, particularly if it has been longer than three months after the second dose. These findings may support the concerns about the possible short-lasting humoral immunity response after the two-dose vaccination schedule, and starting with the older population, booster doses may be needed to help to pursue seropositivity.

The BAU/ml is the conversion factor determined using the World Health Organization international standard code to standardise interlaboratory variability due to different reagents. However, few studies evaluating the antibody response of the Sinovac vaccine have used BAU/ml. Therefore, our study aimed to make qualitative and quantitative

Table 3. Comparison of seropositive and seronegative individuals, in Group 2

		Sars-CoV2 spike IgG antibody positive group $n = 101$ (73.2%)	Sars-CoV2 spike IgG antibody negative group $n = 37$ (26.7%)	*P* value
Age, median (IQR)		71 (67–75.2)	71 (66.2–75)	0.723
Female gender		66 (67.3%)	23 (63.9%)	0.707
Comorbidities	Depression, *n* (%)	15 (15.3%)	4 (11.1%)	0.537
	CVD, *n* (%)	20 (20.4%)	10 (27.8%)	0.364
	HT, *n* (%)	76 (77.6%)	25 (69.4%)	0.334
	DM, *n* (%)	38 (38.8%)	15 (41.7%)	0.742
	Atrial fibrillation, *n* (%)	6 (6.1%)	5 (13.9%)	0.165
	Hypothyroidism, *n* (%)	24 (24.5%)	4 (11.1%)	0.091
	Congestive heart failure, *n* (%)	7 (7.1%)	3 (8.3%)	0.728
	Rheumatological diseases, *n* (%)	2 (2.0%)	7 (19.4%)	0.001*,**
	Malignancy history, *n* (%)	7 (7.1%)	7 (19.4%)	0.055
	Chronic renal disease, *n* (%)	6 (6.1%)	8 (3.8%)	0.701
	Pulmonary diseases, *n* (%)	7 (7.1%)	2 (5.6%)	0.745
Basic ADLs, median (IQR)		6 (5–6)	6 (5–6)	0.331
Instrumental ADLs, median (IQR)		8 (8–8)	8 (8–8)	0.261
MMSE, median (IQR)		28 (25–29)	27.5 (25.7–30)	0.914
Geriatric depression scale score, median (IQR)		2 (0–5)	2.5 (0.7–7)	0.697
Clock drawing test, median (IQR)		6 (2.2–6)	6 (3–6)	0.388
CCI score, median (IQR)		1 (0–1)	1.5 (0–3)	0.011*
CFS score, median (IQR)		3 (3–4)	3 (3–4)	0.729
CFS-frailty, *n* (%)		21 (21.9%)	7 (19.4%)	0.761
MNA-SF score, median (IQR)		14 (13–14)	13.5 (12–14)	0.128
Malnutrition (MNA < 8)		13 (14.1%)	7 (20.6%)	0.379
Number of drugs, median (IQR)		5 (3–7)	5 (3–9)	0.719
Polypharmacy, *n* (%)		20 (55.6%)	55 (56.7%)	0.816
Falls, *n* (%)		15 (16.5%)	7 (21.2%)	0.542
HGS, median (IQR)		19.4 (16.9–26.6)	23.5 (17.7–27)	0.035*
Low HGS, *n* (%)		25 (30.9%)	7 (23.3%)	0.437
Low gait speed, *n* (%)		17 (23.3%)	10 (34.5%)	0.248
Gait Speed, median (IQR)		0.9 (0.8–1.1)	0.85 (0.6–0.9)	0.382
Sars-CoV2 spike IgG serum level U/ml, median (IQR)		2.9 (1.6–6.5)	0.57 (0–0.7)	<0.0001*,**,***
Sars-CoV2 spike IgG serum level BAU/ml, median (IQR)		61.9 (37.0–131.8)	12.4 (0.0–15.2)	<0.0001*,**,***

CVD, Cardiovascular diseases; HT, Hypertension; DM; Diabetes Mellitus; ADL, Activities of daily living; MMSE, Mini-mental State Examination; CFS, Clinical Frailty Scale; MNA-SF, Mini Nutritional Assessment short-form. *Significance at $P < 0.05$. **Significance at $P < 0.01$. ***Significance at $P < 0.001$.

evaluations by giving both U/ml and BAU/ml values. Based on a hospital serological study, young healthcare professionals (mean age: 34.4) who received two doses of CoronaVac, tested after 60 days, had significantly lower sCOVG serum levels compared to those who were tested within 60 days of receiving CoronaVac (111.1 ± 62.63 vs. 237.4 ± 160.4 BAU/ml; $P < 0.001$) [21]. In another study conducted on participants who received two doses of CoronaVac with a mean age of 42.3, the median sCOVG serum level collected after 21–49 days after the second dose of vaccine was found to be 128 BAU/ml [22]. In our study, sCOVG serum level median (IQR) BAU/ml for each group was 79.1 (31.6–166.0), 41.2 (19.8–103.5) and 27.0 (10.9–62.3), respectively. Compared to the few studies conducted in young patients, the lower mean BAU values obtained in a geriatric population may be explained via the factors, particularly immunosenescence, that reduce ageing related antibody response. Since this study's primary aim was not to compare the age groups, more randomised controlled studies comparing older and younger adults are needed to prove this hypothesis.

Vaccine response in the older population is not an entirely well-understood area due to confounders such as immunosenescence (qualitative and quantitative deterioration in immune response due to ageing), frailty and multiple comorbidities [4, 23]. The change of immune organs in older adults is most obvious in thymus; the activity of thymocytes and thymic epithelial cells are reduced, the immune response substances are reduced and therefore immune function is decreased [24]. In addition, the generation of activated B cells and immunoglobulin functionality are important issues [4]. Therefore, immunosenescence may cause alterations in vaccine response in older adults. Frailty is a relatively new concept, providing us an integrative understanding than comorbidities alone of susceptibility to adverse outcomes [25]. Furthermore, recent studies show that immunosenescence is not only a consequence of biological ageing but also a contributor to the variability in

Table 4. Comparison of seropositive and seronegative individuals, in Group 3

		Sars-CoV2 spike IgG antibody positive group $n = 98$ (57.3%)	Sars-CoV2 spike IgG antibody negative group $n = 73$ (42.7%)	*P* value
Age, median (IQR)		75 (68.7–79)	75 (68–81)	0.744
Female gender		63 (64.3%)	4 (54.8%)	0.208
Comorbidities	Depression, *n* (%)	16 (16.3%)	8 (11%)	0.320
	CVD, *n* (%)	29 (29.6%)	19 (26%)	0.608
	HT, *n* (%)	70 (71.4%)	52 (71.2%)	0.978
	DM, *n* (%)	34 (34.7%)	33 (45.2%)	0.164
	Atrial fibrillation, *n* (%)	15 (15.3%)	10 (13.7%)	0.7691
	Hypothyroidism, *n* (%)	8 (18.4%)	10 (13.7%)	0.414
	Congestive heart failure, *n* (%)	11 (11.3%)	3 (4.1%)	0.090
	Rheumatological diseases, *n* (%)	7 (7.1%)	8 (11%)	0.383
	Malignancy history, *n* (%)	14 (14.3%)	3 (4.1%)	0.052
	Chronic renal disease, *n* (%)	2 (2%)	6 (8.2%)	0.074
	Pulmonary diseases, *n* (%)	12 (12.2%)	6 (8.2%)	0.396
Basic ADLs, median (IQR)		6 (5–6)	6 (5–6)	0.232
Instrumental ADLs, median (IQR)		8 (8–8)	8 (6–8)	0.025*
MMSE, median (IQR)		28 (26–29)	28 (24–30)	0.544
Geriatric depression scale score, median (IQR)		2 (1–4)	2 (0–5)	0.769
Clock drawing test, median (IQR)		6 (4–6)	6 (2–6)	0.689
CCI score, median (IQR)		1 (0–2)	1 (0–2)	0.903
CFS score, median (IQR)		3 (3–4)	4 (3–4)	0.028*
CFS-frailty, *n* (%)		13 (13.4%)	23 (34.3%)	0.002*,**
MNA-SF score, median (IQR)		14 (11.5–14)	14 (12–14)	0.926
Malnutrition (MNA < 12)		25 (28.1%)	16 (26.7%)	0.849
Number of drugs, median (IQR)		4 (2–6.5)	5 (2–7)	0.662
Polypharmacy, *n* (%)		54 (55.1%)	46 (63%)	0.299
Falls, *n* (%)		13 (14.3%)	10 (18.9%)	0.469
HGS, median (IQR)		21 (15.0–28.7)	23 (16.1–27.4)	0.419
Low HGS, *n* (%)		26 (33.3%)	21 (42%)	0.321
Low gait speed, *n* (%)		23 (30.7%)	14 (29.2%)	0.860
Gait speed, median (IQR)		0.9 (0.8–1.1)	1 (0.8–1.2)	0.864
Sars-CoV2 spike IgG serum level U/ml, median (IQR)		2.4 (1.37–5.39)	0.5 (0.5–0.59)	<0.0001*,**,***
Sars-CoV2 spike IgG serum level BAU/ml, median (IQR)		52.6 (29.8–117.0)	10.9 (10.9–12.6)	<0.0001*,**,***

CVD, Cardiovascular diseases; HT, Hypertension; DM, Diabetes Mellitus; ADL, Activities of daily living; MMSE, Mini-mental State Examination; CFS, Clinical Frailty Scale; MNA-SF, Mini Nutritional Assessment short-form. *Significance at $P < 0.05$. **Significance at $P < 0.01$. ***Significance at $P < 0.001$.

vulnerability seen with frailty [24, 25]. Current studies have revealed the impact of frailty on other vaccine responses. In a study evaluating pneumococcal vaccine response in older adults, frailty has appeared to be a better predictor of immune response than age alone [26]. Moreover, many studies assessing the impact of frailty on influenza vaccine responses disclosed that frailty was strongly associated with antibody response as a measure of vaccine efficacy [24]. Although there is a growing body of evidence showing the relationship between frailty and vaccine response [15, 26], most of the vaccination studies have not included frail older adults as a major limitation. In the first report of inactivated SARS-CoV-2 vaccine, CoronaVac, tested in older adults (aged ≥ 60 years), used a phase 1/2 study design to assess the safety of two different doses (3 μg and 6 μg) and found similar neutralising antibody responses among adults aged 18–59 years received same doses [27]. However, the most important limitation of this study was evaluating the seroconversion rate solely at days 28 and 56 and having no data on patients' comorbidities or compressive geriatric assessments, including frailty. In another Phase 2 vaccine study conducted on chimpanzee adenovirus vector vaccine developed by AstraZeneca/Oxford University, people aged 60 and older were included, and similar seropositivity on the 28th day was reported in all age groups. Since, this study is not providing a follow up after 28 days and excludes older participants with severe comorbidities and a CFS score of 4 and above, the antibody response in frail older adults has been unknown [28].

In our study, all patients were evaluated in terms of frailty via CFS, a frailty scale most often used for cumulative deficit frailty. Although we observed no relationship between seroconversion and frailty in Groups 1 and 2 after the second dose of vaccine, in Group 3, frailty prevalence with CFS was significantly higher in the seronegative group ($P = 0.002$). Even though most studies in the field of vaccination emphasise that there might be an age-related decrease in antibody response, our study showed no difference between seropositive and negative groups in terms of age, however, frailty seems to be associated with antibody response.

Therefore, frailty might be playing a key role in possible short-lasting humoral immunity response after the two-dose vaccination, clarified after 90 days.

Despite the studies focused on the effect of comorbidities on non-Sars-CoV2 vaccine response [29], there is insufficient data about the impact of comorbidity burden. Although we observed higher scores of CCI in the seronegative subjects of Groups 1 and 2, no relationship was found between seroconversion and CCI in Group 3. In accordance with our findings, in a study with a small group of haemodialysis patients conducted by Torreggiani *et al.*, at the time of the second dose mRNA vaccine (i.e. 3 weeks after the first dose), low neutralising antibody titers were observed in the high CCI scored group [30]. In conclusion, it can be hypothesised that the seroconversion rate, especially in the early period, may be affected by the burden of comorbidity.

The main limitation of our study is the cross-sectional design, which hinders the causal direction of the relationships seen. Another issue that can be considered as a limitation is that the prevaccine antibody status of the patients is not known. Although N-protein IgG measurement is one of the methods that can objectively evaluate whether patients have had COVID-19 before, it was not available to make this measurement for this cross-sectional study. In order to avoid this situation becoming a limitation, patients were evaluated with all suspicious clinical symptoms, contacts with people infected with COVID-19 and rt-PCR and thorax computer tomography results were obtained from the national database since the onset of the pandemic. Patients were excluded from the study in the presence of a suspicious/positive history, symptom or result. In addition, the lack of recurrent antibody measurements of the same patients also causes limitations in the objective evaluation of the real-life course of the antibody response. Furthermore, a follow-up of the patients in terms of COVID-19 infection could provide essential data on the vaccine's effectiveness. Finally, considering that humoral immune response may not be the sole factor affected by immunosenescence, further studies evaluating the effect of cellular immunity on vaccine response may also be needed.

There are also several strengths of the study. This is the first study giving information about the inactive COVID-19 vaccine seroconversion rate proceeding over time in older adults. Another important strength of this study is showing the effect of frailty, an essential component of the assessment of an older individual, on the COVID-19 vaccine response, as a distinctive feature of the study.

In conclusion; to the best of our knowledge, this is the first study assessing antibody response after vaccination with Sars-CoV 2 inactivated vaccine in the Turkish geriatric population. We found that the seropositivity rate was significantly lower in frail geriatric patients after two-dose scheduled vaccination. These findings may support the necessity of a third dose vaccination after two doses of inactive vaccination, especially in the frail older population. Larger sampled randomised controlled trials are needed to confirm the association between frailty and COVID-19 vaccine response.

Supplementary Data: Supplementary data mentioned in the text are available to subscribers in *Age and Ageing* online.

Acknowledgement: Authors especially thank *'Siemens Healthineers'* for their unconditional support. The study's ethics approval has been taken from the Ankara Hacettepe University Department of Medicine clinical research ethics committee, and the decision number is 2021/17-05(KA-21084). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Declaration of Conflicts of Interest: None.

Declaration of Sources of Funding: None.

References

1. https://cdn.who.int/media/docs/default-source/immunization/sage/2021/april/5_sage29apr2021_critical-evidence_sinovac.pdf?fbclid=IwAR2a3iNh5HmsbZ1pCQ7HKcn7U_zHqbG5BzHYfzTw3GUQZpkIyutb3qKXAM8 (29 August, date last accessed).
2. Tanriover MD, Doğanay HL, Akova M *et al.* Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. Lancet 2021; 398: 213–22.
3. Zhang Y, Zeng G, Pan H *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021; 21: 181–92.
4. Gustafson CE, Kim C, Weyand CM, Goronzy JJ. Influence of immune aging on vaccine responses. J Allergy Clin Immunol 2020; 145: 1309–21.
5. Song JY, Cheong HJ, Hwang IS *et al.* Long-term immunogenicity of influenza vaccine among the elderly: risk factors for poor immune response and persistence. Vaccine 2010; 28: 3929–35.
6. Post-hoc Power Calculator. https://clincalc/stats/samplesize.aspx (7 January, date last accessed).
7. Arik G, Varan HD, Yavuz BB *et al.* Validation of Katz index of independence in activities of daily living in Turkish older adults. Arch Gerontol Geriatr 2015; 61: 344–50.
8. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist 1969; 9: 179–86.
9. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12: 189–98.

10. Guigoz Y, Lauque S, Vellas BJ. Identifying the elderly at risk for malnutrition. The mini nutritional assessment. Clin Geriatr Med 2002; 18: 737–57.
11. Yesavage JA, Brink TL, Rose TL *et al.* Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res 1982; 17: 37–49.
12. Rockwood K, Song X, MacKnight C *et al.* A global clinical measure of fitness and frailty in elderly people. CMAJ 2005; 173: 489–95.
13. Özsürekci C, Balcı C, Kızılarslanoğlu MC *et al.* An important problem in an aging country: identifying the frailty via 9 point clinical frailty scale. Acta Clin Belg 2020; 75: 200–4.
14. Cruz-Jentoft AJ, Bahat G, Bauer J *et al.* Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 2019; 48: 16–31.
15. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987; 40: 373–83.
16. Sundararajan V, Henderson T, Perry C, Muggivan A, Quan H, Ghali WA. New ICD-10 version of the Charlson comorbidity index predicted in-hospital mortality. J Clin Epidemiol 2004; 57: 1288–94.
17. https://cdn0.scrvt.com/39b415fb07de4d9656c7b516d8e2d907/b2406e708bf287c5/506564e9207f/Understanding-SARS-CoV-2-IgG-Immunity-Thresholds-and-the-Process-of-Standardization.pdf
18. Erice A, Varillas-Delgado D, Caballero C. Decline of antibody titres 3 months after two doses of BNT162b2 in non-immunocompromised adults. Clin Microbiol Infect 2022; 28: 139.e131–4.
19. Ferenci T, Sarkadi B. RBD-specific antibody responses after two doses of BBIBP-CorV (Sinopharm, Beijing CNBG) vaccine. BMC Infect Dis 2022; 22: 87.
20. Vokó Z, Kiss Z, Surján G *et al.* Nationwide effectiveness of five SARS-CoV-2 vaccines in Hungary-the HUN-VE study. Clin Microbiol Infect 2021; 28: 398–404.
21. Apisarnthanarak A, Nantapisal S, Pienthong T, Apisarnthanarak P, Weber DJ. Healthcare-associated transmission of severe acute respiratory coronavirus virus 2 (SARS-CoV-2) among Thai healthcare personnel who receive 2 doses of a coronavirus disease 2019 (COVID-19) vaccine: a call for considering a booster dose. Infect Control Hosp Epidemiol 2021: 1–2.
22. Yorsaeng R, Suntronwong N, Phowatthanasathian H *et al.* Immunogenicity of a third dose viral-vectored COVID-19 vaccine after receiving two-dose inactivated vaccines in healthy adults. Vaccine 2022; 40: 524–30.
23. Andrew MK, McElhaney JE. Age and frailty in COVID-19 vaccine development. Lancet 2021; 396: 1942–4.
24. McElhaney JE, Verschoor CP, Andrew MK, Haynes L, Kuchel GA, Pawelec G. The immune response to influenza in older humans: beyond immune senescence. Immun Ageing 2020; 17: 10.
25. Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. Lancet 2013; 381: 752–62.
26. Ridda I, Macintyre CR, Lindley R *et al.* Immunological responses to pneumococcal vaccine in frail older people. Vaccine 2009; 27: 1628–36.
27. Wu Z, Hu Y, Xu M *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021; 21: 803–12.
28. Ramasamy MN, Minassian AM, Ewer KJ *et al.* Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. Lancet 2021; 396: 1979–93.
29. Kwetkat A, Heppner HJ. Comorbidities in the elderly and their possible influence on vaccine response. Interdiscip Top Gerontol Geriatr 2020; 43: 73–85.
30. Torreggiani M, Blanchi S, Fois A, Fessi H, Piccoli GB. Neutralizing SARS-CoV-2 antibody response in dialysis patients after the first dose of the BNT162b2 mRNA COVID-19 vaccine: the war is far from being won. Kidney Int 2021; 99: 1494–6.

Received 10 December 2021; editorial decision 2 March 2022

5.6. CoronaVac tem efetividade superior a 70% em idosos na Colômbia, mostra estudo

Um estudo de mundo real conduzido na Colômbia mostrou uma efetividade acima de 70% da CoronaVac para prevenir Covid-19 em idosos no país. Segundo o artigo, menos de 1% dos maiores de 60 anos imunizados com a vacina do Butantan e da Sinovac morreu pela doença. Publicada na The Lancet Healthy Longevity, a pesquisa foi conduzida pelo Ministério da Saúde e Proteção Social da Colômbia e pelas universidades do Norte e de Antioquia.

Os pesquisadores analisaram dados de 2.828.294 de pessoas com mais de 60 anos entre março e outubro de 2021, sendo que metade havia sido vacinada contra o SARS-CoV-2 e a outra não. Como a CoronaVac foi a primeira vacina aprovada para essa população, ela também foi o imunizante mais aplicado (683.284 participantes do estudo), seguido da AstraZeneca (265.730), Pfizer (400.136) e Janssen (64.997).

Os resultados apontaram uma efetividade de 72,1% da CoronaVac contra óbitos na população maior de 60 anos. Menos de 1% dos idosos vacinados com o imunizante do Butantan morreram ou precisaram ser hospitalizados devido à Covid-19. A efetividade geral de todas as vacinas nos idosos foi de 61,6% contra hospitalizações e 79,8% contra mortes.

Em todos os imunizantes estudados, a efetividade para prevenir morte foi 22,6% mais baixa em idosos mais velhos, acima de 80 anos. Isso ocorre não porque as vacinas não funcionam, mas devido ao próprio envelhecimento que reduz as funções imunológicas naturalmente.

"Nossos achados mostram a necessidade de estratégias adicionais de prevenção para a população idosa. Já foi constatado que uma dose de reforço aumenta a resposta imune e, portanto, representa uma potencial solução para a proteção reduzida das vacinas em indivíduos mais velhos", apontam os autores da pesquisa.

Publicado em: 4/4/2022

Effectiveness of COVID-19 vaccines in older adults in Colombia: a retrospective, population-based study of the ESPERANZA cohort

Leonardo Arregocés-Castillo, Julián Fernández-Niño, Maylen Rojas-Botero, Andrés Palacios-Clavijo, Maryory Galvis-Pedraza, Luz Rincón-Medrano, Mariana Pinto-Álvarez, Fernando Ruiz-Gómez, Belem Trejo-Valdivia

Summary

Background Although clinical trials showed that vaccines have high efficacy and safety, differences in study designs and populations do not allow for comparison between vaccines and age groups. The objective of this study was to evaluate the effectiveness of vaccines against COVID-19 in real-world conditions in adults aged 60 years and older in Colombia.

Methods In this retrospective, population-based, matched cohort study, we evaluated the effectiveness of vaccines against COVID-19-related hospitalisation and death in people aged 60 years and older. The full cohort consisted of every person who was eligible to receive a COVID-19 vaccine in Colombia (the ESPERANZA cohort). The exposed cohort consisted of older adults who were fully vaccinated with Ad26.COV2-S, BNT162b2, ChAdOx1 nCoV-19, or CoronaVac, and who did not have a history of confirmed SARS-CoV-2 infection. The unexposed cohort were people aged 60 years and older who had not received any dose of a COVID-19 vaccine during the study period. Participant follow-up was done between March 11, 2021, and Oct 26, 2021. Vaccine effectiveness was estimated as 1–hazard ratio from cause-specific proportional hazards models in the presence of competing risks. We estimated the overall effectiveness of being fully vaccinated, as well as effectiveness for each vaccine, adjusting by main potential confounders. The effectiveness of each vaccine was also assessed by age groups (ages 60–69 years, 70–79 years, and ≥80 years).

Findings 2828294 participants were assessed between March 11 and Oct 26, 2021. For all ages, the overall effectiveness across all assessed COVID-19 vaccines at preventing hospitalisation without subsequent death was 61·6% (95% CI 58·0–65·0, p<0·0001), 79·8% (78·5–81·1, p<0·0001) for preventing death after hospitalisation with COVID-19, and 72·8% (70·1–75·3, p<0·0001) for preventing death without previous COVID-19 hospitalisation. The effectiveness of all vaccines analysed at preventing death after hospitalisation for COVID-19 was 22·6% lower in adults who were aged 80 and older (68·4% [65·7–70·9], p<0·0001) compared with adults aged between 60 and 69 years (91·0% [89·0–92·6], p<0·0001).

Interpretation All vaccines analysed in this study were effective at preventing hospitalisation and death from COVID-19 in fully vaccinated older adults, which is a promising result for the national vaccination programme against COVID-19 in Colombia and in countries where these biologics have been applied. Efforts should be improved to increase coverage among older adults. In addition, given that we observed that the effectiveness of vaccines declined with increasing age, a booster dose is also justified, which should be prioritised for older adults.

Funding Colombian Ministry of Health and Social Protection.

Lancet Healthy Longev 2022; 3: e242–52

Published Online March 21, 2022 https://doi.org/10.1016/S2666-7568(22)00035-6

See Comment page e219

Ministerio de Salud y Protección Social, Bogotá, Colombia (L Arregocés-Castillo DrPH, J Fernández-Niño PhD, M Rojas-Botero PhD, A Palacios-Clavijo MSc, M Galvis-Pedraza MSc, L Rincón-Medrano MSc, M Pinto-Álvarez MSc, F Ruiz-Gómez DrPH, B Trejo-Valdivia PhD); **Departamento de Salud Pública, Universidad del Norte, Barranquilla, Colombia** (J Fernández-Niño); **Facultad de Salud Pública, Universidad de Antioquia, Medellín, Colombia** (M Rojas-Botero); **Centro de Investigación en Nutrición y Salud, Instituto Nacional de Salud Pública de México, Cuernavaca, México** (B Trejo-Valdivia)

Correspondence to: Dr Leonardo Arregocés-Castillo, Ministerio de Salud y Protección Social, Bogotá 110311, Colombia **larregoces@minsalud.gov.co**

Introduction

In addition to high rates of mortality,[1] the COVID-19 pandemic has generated one of the largest social, economic, and health crises in recent history, exacerbating social inequalities between and within countries. With the availability of vaccines with proven safety and efficacy, which were developed in record time, vaccination, together with non-pharmacological measures, became an essential resource to manage the pandemic and to control the spread of the virus.

People who are 60 years and older have been shown to be more likely than younger people to have severe COVID-19, require hospitalisation, and die from COVID-19.[2,3] On Feb 23, 2021, Colombia's national vaccination plan against COVID-19 prioritised vaccinating health-care staff and older adults, with the first doses for this age group given to people aged 80 years and older.

Colombia has a diverse portfolio of vaccines for COVID-19 that were procured mainly on the basis of delivery timelines and the number of doses that producers offered. Colombia has also negotiated supply agreements with five pharmaceutical companies and has adhered to the COVAX procurement mechanism. Among the purchased vaccines are Ad26.COV2-S,

Research in context

Evidence before this study

We searched OVID MEDLINE and MedRxiv on Dec 15, 2021, to identify studies on vaccine effectiveness against COVID-19 in people aged ≥60 years using the search terms "Effectiveness", "COVID-19", "cohort studies", and "Older adults", without date, language, or article type restrictions. In Spain, a large cohort study found that the effectiveness of mRNA COVID-19 vaccines at preventing hospitalisations in institutionalised people aged ≥60 years when fully vaccinated was 88·4% (95% CI 74·9–94·7) and 97·0% (91·7–98·9) for preventing deaths. Similar results were found in Catalonia in a retrospective cohort study that analysed 28 456 nursing home residents who were vaccinated with BNT162b2, which showed an adjusted effectiveness in people who were fully vaccinated of 95·0% (93·0–96·0) for preventing hospital admission and 97·0% (96·0–98·0) for preventing death by COVID-19. Also, a cohort study was conducted in Portugal in 1·8 million people who were aged 65 years and older to evaluate the effectiveness of mRNA vaccines against COVID-19 hospitalisations and deaths. This study found a reduction in the risk of hospitalisation in fully vaccinated older adults of 59·0% (95% CI 32·0–76·0) and a reduction in the risk of death in the same population of 81·0% (73·0–87·0). In a national cohort of people aged 16 years or older in Chile who were immunised with CoronaVac, the subgroup of older adults (aged ≥60 years) who were fully immunised had an adjusted effectiveness of 89·2% (87·6–90·6) for preventing the admission to the intensive care unit, and 86·5% (84·6–88·1) for preventing COVID-19-related death. Other studies that evaluated the effectiveness of vaccines in older adults did not analyse effectiveness in fully vaccinated older adults or did not have a sufficient follow-up time to assess the effectiveness in preventing death. The studies found present methodological differences (including the study population and predominant variant during the period of analysis). Furthermore, published articles focused on the effectiveness of mRNA COVID-19 vaccines. Only few studies were available that compared the effectiveness of various vaccines in older populations, including various vaccine platforms.

Added value of this study

This study presents real-world evidence for the effectiveness of Ad26.COV2-S, BNT162b2, ChAdOx1 nCoV-19, and CoronaVac vaccines, disaggregated by vaccine and age group, in people aged 60 years and older who were fully vaccinated and had no previous confirmed history of SARS-CoV-2 infection. Our results suggest that increasing age reduces the effectiveness of immunisation against COVID-19 and that the vaccine or vaccine platform used might also be associated with reduced effectiveness.

Implications of all the available evidence

In this study we provide evidence of reduced effectiveness of COVID-19 vaccines in people aged 70 years and older in Colombia, after adjusting for many confounders. Other studies have shown that additional doses rapidly increase antibody titters, hence offering an additional dose to those at higher risk of breakthrough infection might increase protection. These results support the use of a booster dose in people aged 60 years and older, regardless of the vaccine used.

BNT162b2, ChAdOx1 nCoV-19, CoronaVac, and mRNA-1273. Vaccine roll-out was done according to availability in Colombia. Although BNT162b2 was the first vaccine to arrive (in February, 2021), CoronaVac was the first to be available in considerable amounts to immunise people aged 60 years and older, therefore it became the most frequently used biologic in this age group. On the contrary, the mRNA-1273 vaccine was the last to be available (in July, 2021), and although some older adults were immunised with this biologic, we did not include these adults in this study because of the short time window available to observe the outcomes of interest.

These vaccines have shown their efficacy and safety in several clinical trials, hence being approved for emergency use in Colombia.[4–8] All of these vaccines showed a wide range in efficacy in preventing a range of COVID-19 severities, but estimates for preventing severe COVID-19 and death were highly uncertain because of the low sample size and low frequency of these outcomes in these trials. Therefore, it is necessary to study the effectiveness of vaccines in uncontrolled real-life conditions, especially in a highly clinically vulnerable population. Colombia's diverse portfolio of vaccines makes it a good setting for evaluating the effectiveness of various vaccines across age groups.

In our study, we aimed to compare the effectiveness of Ad26.COV2-S, BNT162b2, ChAdOx1 nCoV-19, and CoronaVac at preventing hospitalisation and death in people aged 60 years and older who were fully vaccinated and who had no previously confirmed history of SARS-CoV-2 infection (according to the health information systems of Colombia), when the mu variant (B.1.621) was the most prevalent variant in the country. We aimed for evidence generated in this study to inform the comprehensive evaluation of the national vaccination plan against COVID-19 in Colombia and to support decisions made by the Ministry of Health.

Methods

Study design and participants

We conducted a population-based, match-paired cohort study to assess the effectiveness of a complete scheme (ie, all required doses recommended in the manufacturer's guidelines) of COVID-19 vaccination in people aged 60 years and older in Colombia without a confirmed

history of SARS-CoV-2 infection. The data used in this study was collected by the Ministry of Health and Social Protection of Colombia and the National Institute of Health.

The full cohort consisted of every person who was eligible to receive a COVID-19 vaccine in Colombia (the ESPERANZA cohort). In this study, we included people aged 60 years and older, which, in 2021, was projected to be 7 107 914 individuals, according to the National Administrative Department of Statistics. Data for each cohort member was identified by searching each individual's personal identification number (using an anonymised code) across several databases: (1) MiVacuna, which collected sociodemographic data of all people who were eligible to receive a COVID-19 vaccine; (2) PAIWEB, an individual-level vaccine registry; (3) SEGCOVID, which provides follow-up data of confirmed COVID-19 cases; (4) RUAF-ND, which provides a registry of all deaths, including the cause of death; and (5) the high-cost disease registry (Cuenta de Alto Costo), which provides data for patients by disease diagnosis (eg, chronic kidney disease, hypertension, diabetes, cancer, and HIV). These information sources are part of the integrated information system for social protection and satisfy the information quality standards defined in this framework. A flowchart of the database preparation is included in the appendix (p 1).

In this study, we included all people aged 60 years and older who had a complete scheme of COVID-19 vaccination or who had not received any dose of a COVID-19 vaccine. Therefore, the exposed (vaccinated) individuals in the cohort included people who had been immunised with two doses of BNT162b2, ChAdOx1 nCoV-19, or CoronaVac, or with one dose of Ad26.COV2-S; the unexposed (non-vaccinated) individuals in the cohort consisted of people who had not received any dose of a COVID-19 vaccine during the entire study period. We excluded individuals with a confirmed diagnosis of COVID-19 before enrolment in the cohort (we used SEGCOVID to identify those individuals who had a previous confirmed SARS-CoV-2 infection), as well as individuals with heterologous vaccination, and those who were diagnosed, hospitalised, or who had died from COVID-19 within the 14 days following any dose of a vaccine. We also excluded individuals who had incomplete records.

This study complies with the scientific, technical, and administrative regulation for human health research in Colombia, which classifies this study as research without risk as it only used secondary data sources of anonymised information. This study does not therefore require the review or approval of a research ethics committee.

Procedures

Individuals who met the inclusion criteria were first allocated to groups according to their characteristics (ie, potential confounders): sex; age at the time of vaccination (for those vaccinated; in one-year categories); being diagnosed with cancer, diabetes, chronic kidney disease, hypertension, or HIV, which have been shown to be risk factors for severe COVID-19 and mortality from COVID-19;[9,10] health system regime affiliation (contributory or subsidised); and municipality of residence (or department of residence when municipality was missing). For identifying the covariates that should be considered in the matching and adjusting process, we created a directed acyclic graph. These groups were then separated into the exposed and the unexposed groups. Individuals in each group (exposed and unexposed) were assigned a random number from a uniform distribution (from zero to the last number, indicating the last individual in each subgroup that shares the same characteristics given by the matching variables). For each group, random numbers were generated, and a couple was generated by matching each individual of the exposed cohort, as the individual was vaccinated, with an individual randomly selected from the unvaccinated cohort group that had the same characteristics given by the variables used for matching (ie, sex, age at the time of vaccine for those vaccinated [in 1-year categories]; being diagnosed with cancer, diabetes, chronic kidney disease, hypertension, or HIV diagnosis; health system affiliation regime [contributory or subsidised]; and municipality of residence [or department of residence when municipality was missing]). The unvaccinated individual was therefore assigned the same start of follow-up as their vaccinated counterpart. When the unvaccinated individual had an event (hospitalisation or death) before 14 days after the vaccination of their vaccinated counterpart, that unvaccinated individual was replaced by the next unvaccinated individual in the defined order. This process was done iteratively until all the possible pairs were formed within each group. Individuals who were not matched were excluded from the analysis and no one was matched more than once.

For the **directed acyclic graph** see http://dagitty.net/dags.html?id=W1Li3g

See Online for appendix

Outcomes

The primary outcomes of interest in this study were hospitalisations and deaths from COVID-19. We used the definitions recommended by WHO for surveillance of COVID-19.[11] Death from COVID-19 was defined as death that resulted from clinically compatible illness in a probable or confirmed COVID-19 case, unless there was a clear alternative cause of death that could not be related to COVID-19, without a defined period of complete recovery between illness and death.[12]

During the observation period, each individual provided a specific follow-up duration. The follow-up duration for each vaccinated–unvaccinated pair began 15 days after the vaccinated individual in the pair received their last dose (the time period for the vaccine to induce the immune response), the day of the event (hospitalisation or death from COVID-19), or individual censoring. We had three types of right-censoring: people

	Ad26.COV2-S (n=64 997)	BNT162b2 (n=400 136)	ChAdOx1 nCoV-19 (n=265 730)	CoronaVac (n=683 284)	Fully vaccinated with any vaccine (n=1 414 147)	Unvaccinated (n=1 414 147)
Age						
Median, years	65·0 (62·0–70·0)	66·0 (63·0–69·0)	66·0 (63·0–70·0)	72·0 (64·0–80·0)	68·0 (63·0–75·0)	68·0 (63·0–75·0)
Age group						
60–69 years	48 553 (74·7%)	301 302 (75·3%)	188 934 (71·1%)	282 196 (41·3%)	820 205 (58·0%)	820 205 (58·0%)
70–79 years	13 064 (20·1%)	87 630 (21·9%)	69 621 (26·2%)	219 334 (32·1%)	390 304 (27·6%)	390 304 (27·6%)
≥80 years	3380 (5·2%)	11 204 (2·8%)	7175 (2·7%)	181 754 (26·6%)	203 638 (14·4%)	203 638 (14·4%)
Sex						
Male	34 118 (52·8%)	175 260 (43·8%)	128 879 (48·5%)	308 161 (45·1%)	646 265 (45·7%)	64 626 (45·7%)
Female	30 679 (47·2%)	224 876 (56·2%)	136 851 (51·5%)	375 123 (54·9%)	767 882 (54·3%)	767 882 (54·3%)
Affiliation regime to the health system						
Contributory	12 674 (19·5%)	194 066 (48·5%)	104 698 (39·4%)	263 748 (38·6%)	575 558 (40·7%)	575 558 (40·7%)
Subsidised	52 323 (80·5%)	206 070 (51·5%)	161 032 (60·6%)	419 536 (61·4%)	838 589 (59·3%)	838 589 (59·3%)
Comorbidities						
At least one comorbidity	10 725 (16·5%)	102 435 (25·6%)	67 230 (25·3%)	204 302 (29·9%)	384 648 (27·2%)	384 648 (27·2%)
Cancer	325 (0·5%)	4401 (1·1%)	2657 (1·0%)	6833 (1·0%)	14 141 (1·0%)	14 141 (1·0%)
Diabetes	2665 (4·1%)	28 810 (7·2%)	18 601 (7·0%)	51 246 (7·5%)	101 818 (7·2%)	101 818 (7·2%)
Chronic kidney disease	1235 (1·9%)	14 405 (3·6%)	9034 (3·4%)	38 264 (5·6%)	62 222 (4·4%)	62 222 (4·4%)
Hypertension	9880 (15·2%)	93 232 (23·3%)	61 915 (23·3%)	192 686 (28·2%)	357 779 (25·3%)	357 779 (25·3%)
HIV	65 (0·1%)	400 (0·1%)	266 (0·1%)	0	1414 (0·1%)	1414 (0·1%)

Data are median (IQR) or n (%). On account of the matching process, data for vaccinated and unvaccinated cohorts are identical.

Table 1: **Social, demographic, and medical characterisation of study participants by vaccine**

who died from causes other than COVID-19, those who received a booster dose of the vaccine, and those who finished the follow-up and observation period without having presented the outcome of interest. Furthermore, to control for immortal time bias, outcomes that occurred within 14 days of completion of the vaccination scheme were not considered in the analysis.

Statistical analysis

We conducted a descriptive analysis of participants according to the exposure (ie, whether a vaccine was received, and type of vaccine received). Quantitative variables were summarised as median and IQR, and qualitative variables as percentages. We also used Kaplan-Meier estimates and risk tables to describe time to hospitalisation and time to death from COVID-19 across the entire cohort, by age groups (ie, ages 60–69 years, 70–79 years, and ≥80 years), and by vaccine received.

Because events compete over time (ie, hospitalisation with death as a competing risk), we used a competing risk survival analysis by creating a cause-specific Cox regression model with time of the event or the censored time-to-event. From these data we identified three possible outcomes: (1) hospitalisation without subsequent death; (2) death after hospitalisation for COVID-19; and (3) death without a previous record of hospitalisation for COVID-19.

Vaccine effectiveness was estimated as 1−hazard ratio (HR) from cause-specific proportional hazards models in the presence of competing risks. First, we estimated overall effectiveness (across all vaccines) by including a single exposure variable in the model (ie, being vaccinated or unvaccinated) and the matched pairs as a stratum, which allowed us to include the correlation structure among pairs. Thereafter, given that the specific vaccine was not included in the matching process, we estimated the effectiveness for each vaccine and age group in separate models that were adjusted for sex, age, diagnosis of hypertension, diabetes, cancer, kidney disease, or HIV, and health system affiliation regime. Municipality of residence was included as a random intercept (in these models, we did not include the matched pairs as a stratum). For identifying the covariates that should be considered in the matching and adjusting process, we created a directed acyclic graph.

We verified the proportional hazards assumptions by checking the logarithms of the accumulated risks for vaccinated and unvaccinated cohorts, confirming that differences between the curves were constant over time. We also evaluated if the log (survival) and log (t) curves were parallel, and we did two sensitivity analyses for misclassification bias. For the first analysis, we randomly changed a proportion of non-vaccinated individuals to being fully vaccinated with any vaccine, which was done to explore how much vaccine effectiveness would change if we had 25%, 50%, and 75% of registered non-vaccinated individuals as fully vaccinated, which addresses problems such as a lag in reporting vaccination.

	Ad26.COV2-S (n=64 997)	BNT162b2 (n=400 136)	ChAdOx1 nCoV-19 (n=265 730)	CoronaVac (n=683 284)	Fully vaccinated with any vaccine (n=1 414 147)	Unvaccinated (n=1 414 147)
Outcomes						
Hospitalisation without death	17 (<1%)	71 (<1%)	19 (<1%)	555 (<1%)	662 (<1%)	1684 (<1%)
Death after hospitalisation	17 (<1%)	56 (<1%)	15 (<1%)	1061 (<1%)	1149 (<1%)	5413 (<1%)
Death without hospitalisation	2 (<1%)	42 (<1%)	12 (<1%)	511 (<1%)	567 (<1%)	1987 (<1%)
Time of follow-up						
Median, days	88·0 (74·0–94·0)	135·0 (110·0–151·0)	70·0 (60·0–78·0)	146·0 (112·0–171·0)	118·0 (89·0–156·0)	117·0 (87·0–156·0)

Data are n (%) or median (IQR).

Table 2: **Occurrence of main studied outcomes through the study period by vaccine**

The second sensitivity analysis tested the assumption that the 206 607 individuals with missing information on the first vaccination dose (who were excluded from the main analysis), were, in fact, fully vaccinated individuals (appendix pp 2–5).

We used R (version 4.1.0), survival packages 3.2 to 11 (to estimate Kaplan-Meier functions) and risk regression (version 2020.12.08, for the competing risk Cox model) for the analyses.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

The 230-day observation period ran from March 11 to Oct 26, 2021, during which each individual provided a specific follow-up time between the 15th day after completion of the vaccination schedule of the exposed individual in the couple and the day of the individual event or date of censoring. The median time of follow-up was 118 days (IQR 88–156), with longer follow-up time for individuals vaccinated with CoronaVac and shorter time for individuals who received ChAdOx1 nCoV-19 and Ad26.COV2-S. Of the individuals aged 60 years and older who were prioritised to receive a COVID-19 vaccine, we excluded 1 564 092 records because these individuals died before the study observation period, had data quality issues, or met at least one exclusion criterion (appendix p 1). The last consultation of any of these databases was made on Nov 3, 2021.

We analysed a total of 2 828 294 individuals, who were assigned in a 1:1 ratio to the exposed group or to the unexposed group matched by sex, age, health system affiliation status, presence of comorbidities (ie, hypertension, diabetes, chronic kidney disease, HIV, or cancer), municipality of residence, and an approximation of follow-up time.

Hence, because of the matching process, vaccinated and unvaccinated individuals had comparable socio-demographic and clinical characteristics. However, there were differences in these characteristics according to the vaccine administered (table 1).

The cohort consisted of mostly women (1 535 764 [54·3%] of 2 828 294 participants), with a median age of 68 years (IQR 12 years [63–75]). 1 677 178 (59·3%) of 2 828 294 participants were affiliated to the subsidised health system regime. 769 296 (27·2%) had at least one underlying disease identified as a risk factor for becoming seriously ill and dying from COVID-19. As CoronaVac was the first vaccine to be available in the country for mass use, people vaccinated with CoronaVac were older than people who received the other vaccines (table 1).

Table 2 shows the occurrence of each main outcome by vaccine manufacturer. The median follow-up time was 118 days (IRQ 89–156) for all individuals who were fully vaccinated with any vaccine and 117 days (87–156) for all unvaccinated individuals. As Coronovac was the first vaccine available to older adults in Colombia, the longest follow-up time was for this vaccine (median 146 days; IQR 112–171). The shortest follow-up time was for ChAdOx1 nCoV-19 (70 days; 60–78 days), owing to the longer interval needed between doses to finish the schedule.

The risk of hospitalisation and death due to COVID-19 was higher in the unvaccinated cohort, as shown in the Kaplan-Meier survival curves (long rank test p<0·0001; figure 1).

For people aged 60 years and older, the effectiveness of the COVID-19 vaccines in preventing hospitalisations and deaths ranged between 61·6% and 79·8% (table 3). For any vaccine, the effectiveness for preventing hospitalisation without death was 61·6% (95% CI 58·0–65·0, p<0·0001) across all age groups, with the highest effectiveness in adults aged 60–69 years (76·1% ([71·2–80·2], p<0·0001) and the lowest in adults aged 80 years and older (46·9% [38·5–54·1], p<0·0001).

The effectiveness across all vaccines analysed for preventing death after hospitalisation was 79·8% (95% CI 78·5–81·1, p<0·0001) across all ages, with the highest effectiveness in adults aged 60–69 years (91·0% [89·0–92·6], p<0·0001) and the lowest effectiveness in adults aged 80 years and older (68·4% [65·7–70·9],

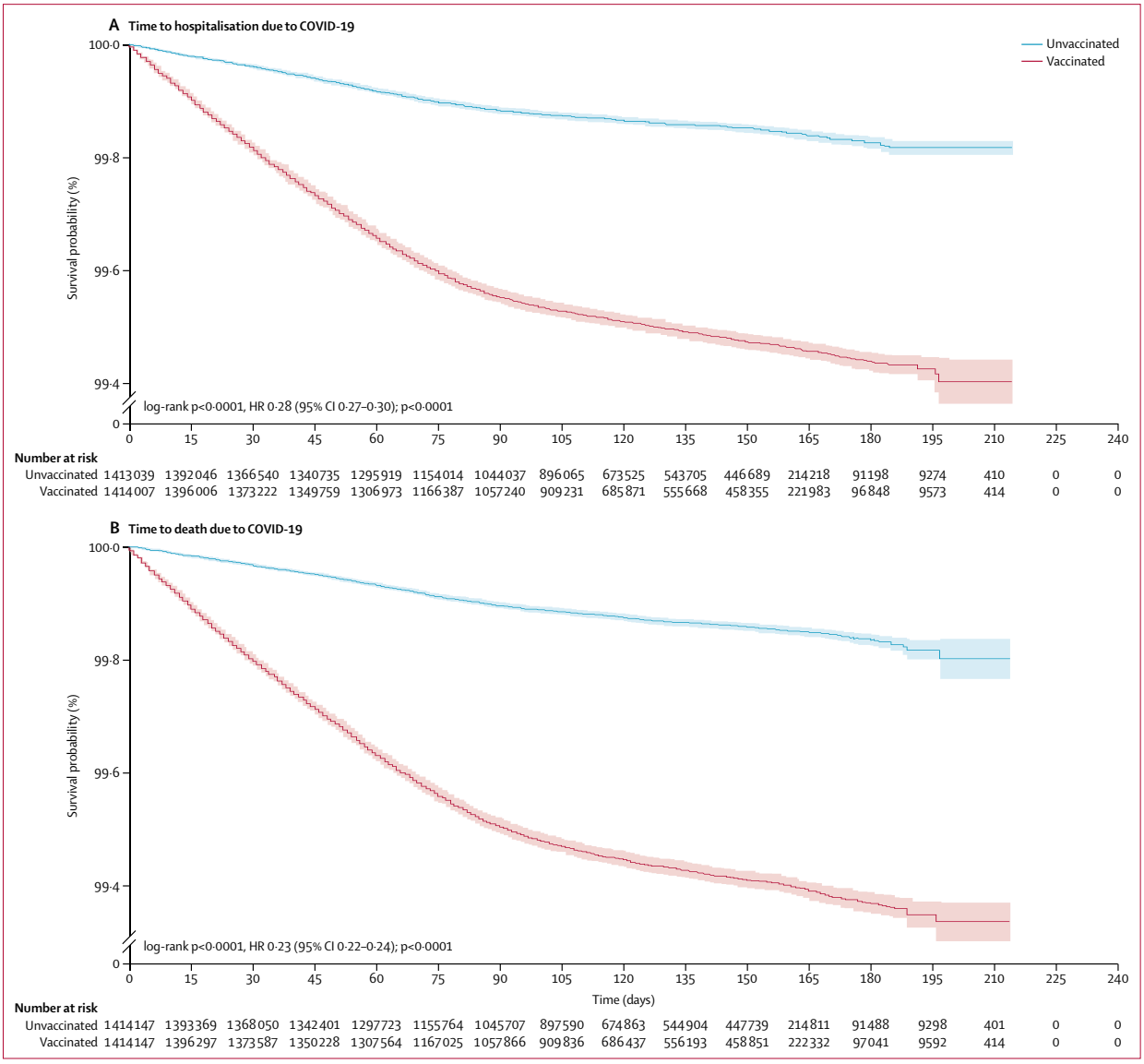


Figure 1: **Kaplan-Meier survival curves for adults aged 60 years and older in Colombia**
(A) Time to hospitalisation due to COVID-19. (B) Time to death due to COVID-19. HR=hazard ratio.

p<0·0001). Finally, the effectiveness across all vaccines for preventing death without hospitalisation was 72·8% (70·1–75·3, p<0·0001), being higher in adults aged 60–69 years (87·6% [83·4–90·7], p<0·0001), reducing to 78·9% (74·6%–82·4%, p<0·0001) in adults aged 70–79 years, and 61·2% (56·3–65·6, p<0·0001) in those aged 80 years and older.

Table 3 also shows the effectiveness of each vaccine by age group. We observed high effectiveness for every vaccine analysed, particularly for preventing death. BNT162b2 and ChAdOx1 nCoV-19 were most effective at preventing all outcomes of interest, with overlapping CIs in people aged 60–79 years. Across all age groups, the effectiveness of BNT162b2 in preventing hospitalisation without death was 83·0% (95% CI 78·4–86·6, p<0·0001), 94·8% (93·3–96·0, p<0·0001) in preventing death after hospitalisation, and 88·3% (84·1–91·4, p<0·0001) in preventing death without hospitalisation. Regarding

ChAdOx1 nCoV-19, across all age groups, effectiveness in preventing hospitalisation without death was 90·8% (85·5–94·2, p<0·0001), 97·5% (95·8–98·5, p<0·0001) in preventing death after hospitalisation, and 93·9% (89·3–96·6, p<0·0001) in preventing death without hospitalisation. For ChAdOx1 nCoV-19, the estimators of effectiveness for each outcome were very similar for the different age groups.

CoronaVac, although showing a high effectiveness in preventing all outcomes, had a lower effectiveness compared with BNT162b2 and ChAdOx1 nCoV-19, with some of the CIs not overlapping (table 3, figure 2). CoronaVac prevented hospitalisation without death in 47·3% (95% CI 41·9–52·3, p<0·0001) of participants, prevented death after hospitalisation in 72·1% (70·1–73·9, p<0·0001) of participants, and prevented death without hospitalisation in 64·9% (61·2–68·2, p<0·0001) of participants. The effectiveness of the CoronaVac vaccine showed a clear tendency to decrease with age. For instance, for adults aged 80 years and older, CoronaVac was 43·4% (34·5–51·2, p<0·0001) effective at preventing hospitalisation without death, 66·3% (63·4–69·0, p<0·0001) effective at preventing death after hospitalisation, and 59·1% (53·8–63·7, p<0·0001) effective at preventing death without hospitalisation.

Finally, regarding Ad26.COV2-S, this vaccine prevented hospitalisation without death in 60·9% (95% CI 36·8–75·8, p<0·0001) of participants, prevented death after hospitalisation in 85·8% (77·1–91·2, p<0·0001) of participants, and prevented death without hospitalisation in 95·5% (82·0–98·9, p<0·0001) of participants. It is possible that the estimates by interval of the effectiveness by age group of this vaccine were imprecise given the small sample size and low numbers of participants vaccinated by this vaccine, so it was not possible to establish if there were significant differences.

In brief, the effectiveness of the vaccines evaluated decreased with age. We found better outcomes among people aged 60–69 years, followed by those aged 70–79 years, with the lowest effectiveness in people aged 80 years and older (figure 2). Although all the vaccines analysed showed a reduction in effectiveness as age increased, this reduction was greater for the CoronaVac vaccine.

For some of the vaccines evaluated, a point estimate of vaccine effectiveness could not be estimated because no outcomes occurred in that age group. In other instances, the CIs were wide because of the low number of people who received that specific vaccine, or the short duration of follow-up. Results of both sensitivity analyses were consistent with results of our primary analyses, suggesting that results are robust and consistent (appendix pp 4–5).

	Hospitalisation without death (95% CI); n=2 828 294	Death after hospitalisation (95% CI); n=2 828 294	Death without hospitalisation (95% CI); n=2 828 294
Any vaccine			
Total	61·6% (58·0–65·0)	79·8% (78·5–81·1)	72·8% (70·1–75·3)
60–69 years	76·1% (71·2–80·2)	91·0% (89·0–92·6)	87·6% (83·4–90·7)
70–79 years	60·8% (54·6–66·2)	85·0% (83·1–86·7)	78·9% (74·6–82·4)
≥80 years	46·9% (38·5–54·1)	68·4% (65·7–70·9)	61·2% (56·3–65·6)
Ad26.COV2-S			
Total	60·9% (36·8–75·8)	85·8% (77·1–91·2)	95·5% (82·0–98·9)
60–69 years	45·8% (7·5–68·2)	85·0% (69·9–92·5)	95·0% (64·2–99·3)
70–79 years	77·9% (31·1–92·9)	88·6% (72·5–95·3)	93·4% (52·7–99·1)
≥80 years	..	81·9% (51·7–93·2)	..
BNT162b2			
Total	83·0% (78·4–86·6)	94·8% (93·3–96·0)	88·3% (84·1–91·4)
60–69 years	84·0% (77·8–88·5)	94·0% (91·4–95·8)	88·1% (81·3–92·4)
70–79 years	81·3% (72·5–87·3)	96·2% (93·9–97·6)	89·9% 82·9–94·1)
≥80 years	79·3% (49·9–91·4)	92·7% (85·4–96·4)	83·4% (66·6–91·7)
ChAdOx1 nCoV-19			
Total	90·8% (85·5–94·2)	97·5% (95·8–98·5)	93·9% (89·3–96·6)
60–69 years	88·4% (79·4–93·5)	98·3% (95·4–99·4)	93·7% (84·9–97·4)
70–79 years	92·4% (84·0–96·4)	96·6% (93·7–98·2)	95·7% (88·4–98·4)
≥80 years	..	98·0% (85·7–99·7)	86·5% (57·9–95·7)
CoronaVac			
Total	47·3% (41·9–52·3)	72·1% (70·1–73·9)	64·9% (61·2–68·2)
60–69 years	63·4% (52·8–71·6)	83·3% (78·5–87·1)	82·5% (73·7–88·3)
70–79 years	44·0% (34·5–52·2)	78·1% (75·1–80·7)	70·7% (64·4–76·0)
≥80 years	43·4% (34·5–51·2)	66·3% (63·4–69·0)	59·1% (53·8–63·7)

All estimators were statistically significant (p<0·0001). The results for any vaccine were obtained from a cause-specific Cox regression model, in which each pair of vaccinated and unvaccinated individuals represented a stratum within the model, according to the study design. The results for each vaccine were obtained from multivariate cause-specific Cox regression models, which were adjusted by age, sex, affiliation regime to the Colombian health system, cancer, diabetes, hypertension, kidney disease, and HIV, with a random effect for municipality of residence. The reference group corresponds to people who have not received any dose of the COVID-19 vaccine.

***Table 3*: Effectiveness of vaccines in preventing hospitalisation and death due to COVID-19 in adults aged 60 years and older in Colombia**

Discussion

We conducted a retrospective, national-cohort study to evaluate the effectiveness of vaccines against COVID-19 in people aged 60 years and older in Colombia. We included individuals who received the complete vaccination schedule between March 11 and Oct 26, 2021, when the mu variant was the most prevalent in the country. The results of this study confirm the high effectiveness of the available vaccines in Colombia for preventing hospitalisations and deaths due to COVID-19. These results are congruous with those reported by vaccine manufacturers, obtained from controlled clinical trials.[4–7]

For all vaccines studied, we found an overall effectiveness of 61·6% for preventing hospitalisation without subsequent death, 79·8% for preventing death after hospitalisation, and 72·8% for preventing death without previous hospitalisation. These results are similar to those found in similar populations in other countries. A study[13] done in fully vaccinated older long-term care residents in Spain found that the effectiveness of mRNA COVID-19 vaccines in preventing hospitalisations was 88·4% (95% CI 74·9–94·7) and 97·0% (91·7–98·9) for preventing death. Similarly, a

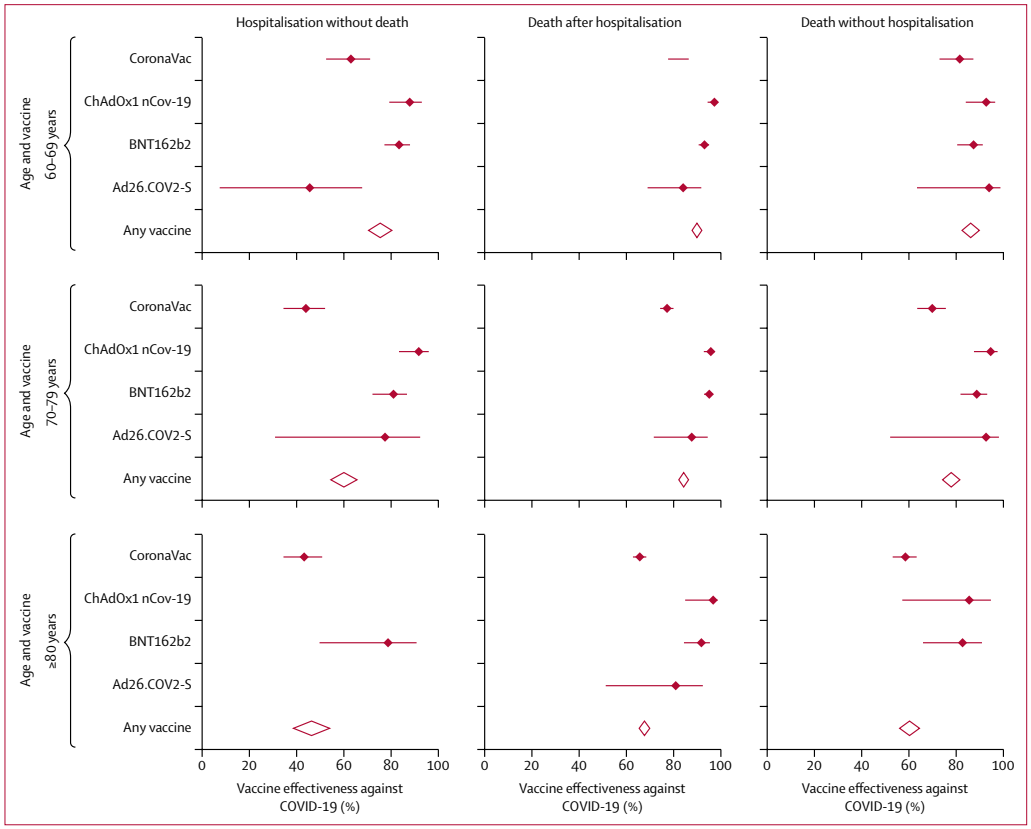


Figure 2: **Forest plot of vaccine effectiveness at preventing hospitalisation and death due to COVID-19 in adults aged 60 years and older in Colombia**

retrospective cohort study[14] in Catalonia, which analysed 28 456 nursing home residents who were vaccinated with BNT162b2, had an adjusted effectiveness in those who were fully vaccinated of 95·0% (93·0–96·0) for preventing hospital admission and 97·0% (96·0–98·0) for preventing death. Also, a cohort study[15] done in Portugal evaluated the effectiveness of mRNA vaccines against COVID-19 hospitalisations and deaths in 1·8 million people who were aged 65 years and older and found a reduction in the risk of hospitalisation (59·0% [32·0–76·0]) and death (81·0% [73·0–87·0]) in fully vaccinated older adults.

In our study, vaccine effectiveness was negatively correlated with older age, regardless of the vaccine used, but not all vaccine platforms were affected the same. Among the vaccines included in this study, viral vector and mRNA vaccines were associated with higher effectiveness with increasing age than inactivated virus vaccines. Colombia used CoronaVac for the prioritised older population because it was the first vaccine to be available in sufficient quantities. Although mRNA vaccines have a higher vaccine effectiveness than CoronaVac, waiting several months for them to become available in Colombia would have been an unnecessary risk. This rationale had already been suggested by other authors, in which early vaccination with less effective biologics outweighed the advantages of waiting for other, more effective vaccines to become available.[16]

In Chile, one study[17] analysed a national cohort of people aged 16 years or older who were immunised with CoronaVac. In the subgroup of older adults (aged 60 years and older) who were fully immunised, CoronaVac was 89·2% (95% CI 87·6–90·6) effective at preventing older adults from admission to the intensive care unit, and 86·5% (84·6–88·1) effective at preventing COVID-19-related death. Other studies that evaluated the effectiveness of vaccines in older people did not analyse vaccine effectiveness in older adults who were fully vaccinated or did not have a sufficient follow-up time to assess the effectiveness of preventing death.[18]

These studies present methodological differences, including the study population and predominant variant during the period of analysis, thus the above studies should be compared against our results carefully.

Furthermore, these other studies focused on mRNA COVID-19 vaccines.[5,8,13,15] Only a small number of studies are available for comparing the effectiveness of various vaccines in older populations, including various vaccine platforms.[18,19]

We observed that the effectiveness across vaccines in preventing death after hospitalisation decreased by 22·6% and decreased by 26·4% in preventing death without previous hospitalisation for those aged 80 years and older compared with adults aged 60–69 years. Possible explanations for this lower effectiveness include the greater probability of pre-existing conditions in older people, in conjunction with age-related frailty[20] and immunosenescence, which can cause poor responses to vaccination.[21] These results are congruent with those presented in other studies.[22,23]

Older adults have already been identified as the population with the highest risk of severe illness and death from COVID-19. Our findings show the need for additional prevention strategies for this age group. A booster dose has been found to increase the immune response, and therefore represents a potential solution to the decreased effectiveness of vaccines in older people.[24] On the basis of our findings, Colombia started offering booster doses to people aged 50 years and older from early October, 2021.

The mu variant was predominant in Colombia throughout the observation period. Although there is no information on genomic sequencing in instances of breakthrough infections, the high observed effectiveness of vaccines indirectly suggests that all vaccines used in Colombia confer adequate protection against this variant and other variants circulating during the study period. However, our study was not able to assess the effectiveness of vaccines for any specific variant of SARS-CoV-2, given that the evaluated outcomes did not distinguish the variant involved in the clinical course of the patients. Therefore, our results could not be totally extrapolated to other contexts in which other variants are predominant.

The differences in self-selection to access the application of the vaccines could be a source of confounding in this study (eg, an unvaccinated adult might also be less likely to wear a mask or take precautions, thereby exposing themselves to greater risk of infection, which could contribute to the observed differences). However, we consider that this bias, if present, would have only a small effect on the estimations. This is because, first, according to previous surveys of vaccination intention in Colombia, older adults had a high willingness to get vaccinated. Second, vaccination coverage in older adults at the end of December, 2021, was above 90%, which confirms that vaccine hesitancy was not an issue in this age group. Finally, according to international evidence, one of the factors associated with refusal to be vaccinated is social class. This factor was partly controlled for in this study by adjusting for affiliation regime to the health system, which is a strong proxy indicator of social class in Colombia.

However, as any cohort study, this different propensity to be exposed (vaccinated in this instance) could be related to unobserved (or unobservable) variables that could affect the estimates. It should be considered that this might mean that unvaccinated people did not get vaccinated because of individual characteristics that are also associated with a higher risk of infection, such as lower self-care, which could lead to overestimating the effect.

Thus, because there are many factors that influence whether people are vaccinated, and the bias could either lead to overestimation or underestimation of the observed effectiveness estimates, this is a limitation of the study. However, this limitation is minimised because of the high willingness and rate of vaccination in Colombia, as well as the study indirectly controlling for social class in this age group and by the matching process in the design.

We identified delays in reporting to the individual-level vaccination registry system (PAIWEB), which introduced a high probability of misclassification bias. The vaccinated population who had not been registered as such is likely to have reduced the differences in risk for outcomes of interest between the exposed cohort and the unexposed cohort. This bias tended to underestimate the observed effectiveness, so we believe that the presented estimators are lower than the actual effectiveness values. We conducted a sensitivity analysis in which we randomly changed a proportion of non-vaccinated individuals as being fully vaccinated with any vaccine, equal to the estimated proportion of people fully vaccinated but not registered as such (appendix p 4). By the cutoff date (Oct 26), approximately 24% of those vaccinated had not been registered, although people aged 60 years and older were more likely to be reported because they were the first to be vaccinated. Results of this analysis suggest a higher vaccine effectiveness when individuals in the unexposed cohort were randomly assigned as being exposed, suggesting that our observed effectiveness was underestimated.

Another limitation of our study was that 206 607 people had a record labelled as a second dose, but without the first dose being registered. These records were excluded from our analysis. A sensitivity analysis that included all records, assuming that all individuals were fully vaccinated, showed similar results to our primary analysis and did not show a significant increase in overall vaccination effectiveness, suggesting that these records might correspond to mislabelling and that our estimates are robust.

Another limitation of this study is the short follow-up period for people vaccinated with Ad26.COV2-S and ChAdOx1 nCoV-19, which led to a small number of observed events, especially for Ad26.COV2-S, and therefore lower precision of the estimators. For future studies, we

recommend repeating this analysis with a longer follow-up period. Moreover, it was not possible to control for other possible confounding variables, such as frailty or residing in nursing homes, among others. So, it is possible that our results have some residual confounding.

Our study had several strengths. To the best of our knowledge, this study is the first investigation to analyse and report the effectiveness of four different vaccines against COVID-19 in older adults disaggregated by age group. There have only been few publications that compare inactivated virus vaccines with mRNA and viral vector vaccines in the same country in high-risk populations. This makes our study relevant for countries where inactivated virus vaccines were widely used in older populations to act towards better protecting those at higher risks of severe illness from COVID-19. In addition, the competing risk analysis we used had the advantage that, instead of estimating a separate model for each outcome, it fitted a joint model for all outcomes. This analysis is especially useful since outcomes (hospitalisation and death from COVID-19 in this instance) might occur at different points in time, especially when one of them might have affected the censoring and risk of the other.[25] Also, we conducted several sensitivity analyses, which allowed us to confirm the robustness of our findings (appendix pp 4–5).

Our study generates solid evidence on vaccination effectiveness in older people in Colombia and might inform the administration of these vaccines in older populations across the world and decision-making that considers risk stratification approaches to target the most vulnerable people according to their demographic and epidemiological characteristics.

Contributors

LA-C and JF-N conceptualised the effectiveness evaluation of COVID-19 vaccines strategy. LA-C, MR-B, and JF-N conceptualised the design and statistical analysis plan for the present study. LR-M built the dataset. BT-V advised the study design and the statistical analysis. AP-C and LR-M cleaned the data. AP-C and MR-B did the statistical analysis. LA-C, JF-N, LR-M, MR-B, and MG-P verified the data underlying the study. LA-C, MR-B, and MP-Á wrote the first draft. All authors reviewed and discussed the results and approved the final version of the manuscript. JF-N, AP-C, and LA-C accessed and verified the data. All authors agree with the viewpoints expressed in the article and contributed to the review and editing of the manuscript.

Declaration of interests

FR-G, LA-C, and JF-N are members of the Colombian COVID-19 vaccine advisory committee. All other authors declare no competing interests.

Acknowledgments

This study was conducted by the Ministry of Health and Social Protection of Colombia. Workers were from the Directorate of Medicines and Health Technologies, and Directorate of Epidemiology and Demography. Funding from the Ministry was given indirectly through the wage of and payments to the statistician of the project.
In Colombia, public data from health information systems at the individual level are managed and safeguarded by the Ministry of Health and Social Protection. In the case of information on vaccination and covariates at the individual level, these will be publicly available once the technical and regulatory requirements of the Ministry have been adjusted, which is currently in process. The databases are not yet available to the public because authorisation from the Ministry has not been obtained. However, according to regulations, these data can be requested by researchers who ask for the information, who must also send the objective of the investigation, a plan of analysis, and a publication plan to the Ministry of Health (https://www.minsalud.gov.co/atencion/Paginas/Solicitudes-sugerencias-quejas-o-reclamos.aspx). Each request will be evaluated in accordance with the regulations and legal requirements defined in the country.

References

1 Johns Hopkins University & Medicine. Coronavirus resource center. Global map. 2021. https://coronavirus.jhu.edu/map.html (accessed Feb 3, 2022).
2 Ho FK, Petermann-Rocha F, Gray SR, et al. Is older age associated with COVID-19 mortality in the absence of other risk factors? General population cohort study of 470,034 participants. *PLoS One* 2020; **15:** e0241824.
3 Chen Y, Klein SL, Garibaldi BT, et al. Aging in COVID-19: vulnerability, immunity and intervention. *Ageing Res Rev* 2021; **65:** 101205.
4 Sadoff J, Gray G, Vandebosch A, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. *N Engl J Med* 2021; **384:** 2187–201.
5 Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 2020; **383:** 2603–15.
6 Falsey AR, Sobieszczyk ME, Hirsch I, et al. Phase 3 safety and efficacy of AZD1222 (ChAdOx1 nCoV-19) Covid-19 vaccine. *N Engl J Med* 2021; **385:** 2348–60.
7 Tanriover MD, Doğanay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *Lancet* 2021; **398:** 213–22.
8 Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* 2021; **384:** 403–16.
9 Cifuentes MP, Rodriguez-Villamizar LA, Rojas-Botero ML, et al. Socioeconomic inequalities associated with mortality for COVID-19 in Colombia: a cohort nationwide study. *J Epidemiol Community Health* 2021; **75:** 610–15.
10 Nasiri MJ, Haddadi S, Tahvildari A, et al. COVID-19 clinical characteristics, and sex-specific risk of mortality: systematic review and meta-analysis. *Front Med* 2020; **7:** 459.
11 WHO. Public health surveillance for COVID-19. Interim guidance. 2020. https://www.who.int/publications/i/item/who-2019-nCoV-surveillanceguidance-2020.8 (accessed Feb 3, 2022).
12 Pan American Health Organization. Case definitions for COVID-19 surveillance. 2020. https://www.paho.org/en/case-definitions-covid-19-surveillance-16-december-2020 (accessed Feb 3, 2022).
13 Mazagatos C, Monge S, Olmedo C, et al. Effectiveness of mRNA COVID-19 vaccines in preventing SARS-CoV-2 infections and COVID-19 hospitalisations and deaths in elderly long-term care facility residents, Spain, weeks 53 2020 to 13 2021. *Euro Surveill* 2021; **26:** 2100452.
14 Cabezas C, Coma E, Mora-Fernandez N, et al. Associations of BNT162b2 vaccination with SARS-CoV-2 infection and hospital admission and death with covid-19 in nursing homes and healthcare workers in Catalonia: prospective cohort study. *BMJ* 2021; **374:** n1868.
15 Nunes B, Rodrigues AP, Kislaya I, et al. mRNA vaccine effectiveness against COVID-19-related hospitalisations and deaths in older adults: a cohort study based on data linkage of national health registries in Portugal, February to August 2021. *Euro Surveill* 2021; **26:** 2100833.
16 Ahuja A, Athey S, Baker A, et al. Preparing for a pandemic: accelerating vaccine availability. National Bureau of Economic Research. 2021. https://www.nber.org/papers/w28492 (accessed Feb 3, 2022).
17 Jara A, Undurraga EA, González C, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. *N Engl J Med* 2021; **385:** 875–84.
18 Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. *BMJ* 2021; **373:** n1088.

19 Cerqueira-Silvaa T, de Araújo V, Boaventura V, et al. Influence of age on the effectiveness and duration of protection of Vaxzevria and CoronaVac vaccines: a population-based study. *Lancet Reg Health Am* 2022; **6:** 100154.

20 Hoogendijk EO, Afilalo J, Ensrud KE, Kowal P, Onder G, Fried LP. Frailty: implications for clinical practice and public health. *Lancet* 2019; **394:** 1365–75.

21 Crooke SN, Ovsyannikova IG, Poland GA, Kennedy RB. Immunosenescence and human vaccine immune responses. *Immun Ageing* 2019; **16:** 25.

22 Ranzani OT, Hitchings MDT, Dorion M, et al. Effectiveness of the CoronaVac vaccine in the elderly population during a gamma variant-associated epidemic of COVID-19 in Brazil: a test-negative case-control study. *BMJ* 2021; **374:** n2015.

23 Li M, Yang J, Wang L, et al. A booster dose is immunogenic and will be needed for older adults who have completed two doses vaccination with CoronaVac: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *medRxiv* 2021; published online July 21. doi.org/10.1101/2021.05.19.21257472 (preprint).

24 Flaxman A, Marchevsky N, Jenkin D, et al. Tolerability and immunogenicity after a late second dose or a third dose of ChAdOx1 nCoV-19 (AZD1222). *SSRN* 2021; published online June 28. https://doi.org/10.2139/ssrn.3873839 (preprint).

25 Austin PC, Lee DS, Fine JP. Introduction to the analysis of survival data in the presence of competing risks. *Circulation* 2016; **133:** 601–09.

5.7. Terceira dose da CoronaVac eleva proteção em idosos para 98% contra casos graves e mortes, aponta estudo

Uma pesquisa conduzida pela Universidade de Hong Kong mostrou que a dose de reforço da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, tem uma efetividade de 98% para proteger idosos contra casos graves e mortes por Covid-19, mesmo durante o surto da variante ômicron do SARS-CoV-2. Entre aqueles com mais de 80 anos, a terceira dose protegeu 96,6% contra o mesmo desfecho. O estudo foi publicado na plataforma de preprints MedRxiv.

Os cientistas analisaram dados de casos leves e moderados (5.474), casos graves (5.294) e mortes (4.093) relacionados à Covid-19, ocorridos entre dezembro de 2021 e março de 2022, e investigaram a eficácia de duas e três doses da CoronaVac e da Pfizer, os imunizantes mais utilizados em Hong Kong. Com duas doses, a CoronaVac protegeu 74% dos pacientes maiores de 60 anos contra doença grave e óbito, nível semelhante ao observado na Pfizer. A terceira dose da vacina do Butantan elevou essa proteção para 98%.

Em adultos mais jovens, entre 20 e 59 anos, a proteção com duas doses de CoronaVac contra casos graves foi de 91,7% e, contra mortes, 94%. Já com a dose de reforço, o imunizante preveniu a doença grave em 98,5% dos indivíduos (em relação à mortalidade após terceira dose, não foram obtidos dados suficientes para estimativa).

A ômicron fez a Covid-19 disparar em Hong Kong em janeiro, resultando em 649.454 casos confirmados e quase cinco mil mortes até meados de março. A cobertura vacinal, no entanto, ainda está abaixo do ideal, principalmente no grupo de idosos: 66% das pessoas na faixa etária de 70 a 79 anos e apenas 37% acima de 80 anos receberam as duas doses. Os níveis observados para a terceira dose são ainda mais baixos, com 30% e 10%, respectivamente.

"Nosso estudo ressalta que a terceira dose é importante e deve ser priorizada, principalmente para os idosos, pois fornece uma proteção adicional contra a forma grave da Covid-19", afirmam os autores no artigo. Eles acrescentam que Hong Kong está reunindo esforços para ampliar a cobertura vacinal nesse público, estendendo o horário de funcionamento das clínicas de imunização e o envio de equipes de vacinação para casas de repouso, conjuntos habitacionais e para pessoas com mobilidade reduzida.

Os resultados de Hong Kong reforçam as descobertas de outros estudos de efetividade feitos no mundo, que comprovam que a CoronaVac garante a proteção de idosos.

Publicado em: 24/3/2022

Vaccine effectiveness of two and three doses of BNT162b2 and CoronaVac against COVID-19 in Hong Kong

Martina E. McMenamin[1], Joshua Nealon[1], Yun Lin[1], Jessica Y. Wong[1], Justin K. Cheung[1], Eric H. Y. Lau[1,2], Peng Wu[1,2], Gabriel M. Leung[1,2], Benjamin J. Cowling[1,2]

Affiliations:

1. World Health Organization Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China
2. Laboratory of Data Discovery for Health, Hong Kong Science and Technology Park, Hong Kong Special Administrative Region, China

Corresponding authors:

Joshua Nealon (jnealon@hku.hk) and Ben Cowling (bcowling@hku.hk)

Running head: COVID-19 vaccine effectiveness in Hong Kong

Word count (abstract): 249

Word count (main text): 3,484

Abstract

Background: Hong Kong maintained extremely low circulation of SARS-CoV-2 until a major community epidemic of Omicron BA.2 starting in January 2022. Both mRNA BNT162b2 (BioNTech/Fosun Pharma) and inactivated CoronaVac (Sinovac) vaccines are widely available, however coverage has remained low in older adults. Vaccine effectiveness in this predominantly infection-naïve population is unknown.

Methods: We used individual-level case data on mild/moderate, severe/fatal and fatal hospitalized COVID-19 from December 31, 2021 to March 8, 2022, along with census information and coverage data of BNT162b2 and CoronaVac. We used a negative binomial model, adjusting for age and calendar day to estimate vaccine effectiveness of one, two and three dose schedules of both vaccines, and relative effectiveness by number of doses and vaccine type.

Findings: A total of 12.7 million vaccine doses were administered in Hong Kong's 7.3 million population, and we analyzed data from confirmed cases with mild/moderate (N=5,474), severe/fatal (N=5,294) and fatal (N=4,093) COVID-19. Two doses of either vaccine protected against severe disease and death, with higher effectiveness among adults ≥60 years with BNT162b2 (VE: 88.2%, 95% confidence interval, CI: 84.4%, 91.1%) compared to CoronaVac (VE: 74.1%, 95% CI: 67.8%, 79.2%). Three doses of either vaccine offered very high levels of protection against severe outcomes (VE: 98.1%, 95% CI: 97.1%, 98.8%).

Interpretation: Third doses of either BNT162b2 or CoronaVac provide substantial additional protection against severe COVID-19 and should be prioritized, particularly in older adults who received CoronaVac primary schedules. Longer follow-up is needed to assess persistence of different vaccine platforms and schedules.

45 **Funding**: COVID-19 Vaccines Evaluation Program, Chinese Center for Disease Control and
46 Prevention

INTRODUCTION

Hong Kong Special Administrative Region of China (Hong Kong; population 7.3 million) has pursued a COVID-19 elimination strategy since January 2020 involving stringent social distancing measures, border entry restrictions, isolation of cases and quarantine of close contacts, and the use of personal protective measures.[1] Consequently, the disease had been largely controlled through December 2021 with four previous epidemic waves resulting in a total of 12,606 cases (<2 per 1,000) and 207 deaths (<3 per 100,000). Since February 2021, both inactivated (Sinovac; CoronaVac) and mRNA (BioNTech/Fosun Pharma; BNT162b2) vaccines have been widely available with residents offered the choice of either. However, by January 2022, two-dose vaccine coverage had only reached 46% in older adults 70-79 years of age and 18% in those aged ≥80 years.[2]

A major community epidemic of COVID-19 Omicron variant (B.1.1.529) lineage BA.2 began in early January 2022, resulting in 649,454 laboratory confirmed cases, 313,127 cases reported by rapid antigen tests and nearly 5,000 deaths to March 17, 2022.[2,3] Vaccination coverage has since risen steadily but remains low in the most vulnerable, with two-dose coverage at 66% and 37% in 70-79 and ≥80 year olds respectively as of March 17, 2022. Third vaccine doses were recommended first for priority groups and then for the general public on 1 January 2022, to be given six months after the second dose.[4,5] Third-dose uptake has been highest in the 40-59y age group (46% as of March 17, 2022) and lower in older adults (30% in 70-79 year olds; 10% in those ≥80). Efforts to increase vaccine uptake in older and high-risk groups are underway, including reducing the duration between first and second doses for care home residents, extending vaccination clinic operating hours and deployment of vaccine outreach teams to care homes, housing estates and to residents with limited mobility.[6,7]

International data has shown vaccination with BNT162b2 reduces the frequency of severe outcomes, and to a lesser extent, infection for variants circulating prior to Omicron.[8–15] Waning of protection has been observed in multiple contexts, in particular against infection,[16–18] and recent studies have provided early indications of reduced effectiveness of BNT162b2 against the Omicron variant.[19–21] Evidence on vaccine performance against the more transmissible Omicron subvariant BA.2 remains very limited, as is data on the performance of the inactivated CoronaVac vaccine.[22] Limited observational evidence suggests strong and durable protection against severe disease and death, with transient protection against milder symptomatic disease.[23–26] With a largely infection-naïve population and two COVID-19 vaccines in widespread use, Hong Kong represents a unique environment for monitoring vaccine effectiveness (VE) against Omicron BA.2. In this study we estimated VE of one, two and three doses of BNT162b2 and CoronaVac, their relative effectiveness, and the additional protection offered by third doses against mild/moderate infections, severe/fatal disease and death.

METHODS

Study design and population

We assessed VE of the BNT162b2 and CoronaVac vaccines using an ecological study design, which has been previously employed to provide estimates of VE in Israel.[27] The study population consisted of residents of Hong Kong aged 20 years and over, where the population with zero, one, two or three doses of either vaccine at risk at a given time was derived using detailed data from the vaccination programme and population census. Information on all laboratory-confirmed SARS-CoV-2 cases in Hong Kong from December 31, 2021 to March 8, 2022 was obtained from nationwide individual level surveillance data provided by the

Centre for Health Protection and linked to clinical outcome data provided by the Hospital Authority.

Ethical approval

This project received approval from the Institutional Review Board of the University of Hong Kong.

Infections and outcomes

Extensive PCR testing for SARS-CoV-2 is conducted in public hospitals, community test centres and private laboratories in Hong Kong. Testing is free-of-charge or available at low cost, and required for those who exhibit COVID-19 like symptoms, or following contact tracing based on exposure history or residential location. Regular screening is also required of certain professions, in particular those working with older adults or vulnerable persons. Positive rapid test results have been recognised as confirmed infections since February 25, 2022 and included in official case counts from March 7, 2022. Data on all laboratory-confirmed cases between December 31, 2021 and March 8, 2022 were extracted and cases classified as 'imported', i.e. detected in on-arrival quarantine, were excluded due to their non-representative SARS-CoV-2 exposure and vaccination histories. Sequencing of a subset of cases each day indicates that fewer than 1% of cases and deaths during the fifth wave have occurred with the Delta variant, with the remaining infections attributed to the Omicron BA.2 lineage.

Hong Kong has an advanced public and private healthcare system whereby private clinics comprise most primary care and government hospitals provide approximately 90% of hospital medical services at very low cost to patients.[28] Up until mid-February 2022, all laboratory-

confirmed COVID-19 cases were admitted to hospitals for isolation and standardized clinical management, regardless of symptom presentation, with their hospitalization records stored in the data system managed by the Hospital Authority. After mid-February 2022, due to the large number of incident cases, hospitalisation was reserved for patients with more severe disease, and milder cases were required to isolate at dedicated government quarantine facilities or at home. In the Hospital Authority data system, records of patients' test results, medication and condition changes were documented and integrated into a centralized database from which we extracted relevant information on those experiencing mild/moderate disease prior to February 16, 2022 and severe disease and death at any time. We excluded those with conflicting information in the database, i.e. persons with a worst recorded condition of 'mild' but also experiencing a fatal outcome within hospital. Severe disease was defined as any severe, critical or fatal COVID-19 case (definitions for each in Appendix).

Population uptake of COVID-19 vaccines

Data on the estimated population size at the end of 2021 by age and sex were obtained from the Census and Statistics Department of the Hong Kong Special Administrative Region Government. Data on the number of persons vaccinated with either the BNT162b2 or CoronaVac vaccines in Hong Kong each day since February 22, 2021 are available in a national vaccination database provided by the Department for Health. Data on all vaccinations that had occurred up to March 8, 2022, including vaccinee age and the type and date of receipt of each dose of vaccine, were extracted on March 10, 2022. Vaccination information for all cases in the surveillance data was cross checked with Hospital Authority records and any cases with discrepancies were excluded.

Those who received vaccines other than BNT162b2 or CoronaVac, or who received a mixed primary series of one dose of BNT162b2 and one dose of CoronaVac, were excluded from the analysis. In addition, for the purposes of this analysis we also exclude those who switched vaccine platform after the second dose, that is, those who received two doses of CoronaVac and a third dose of BNT162b2 and those who have received a primary series of BNT162b2 and a third dose of CoronaVac. Cases with known prior COVID-19 infection were also excluded.

Statistical analysis

Incidence rates were calculated according to the number of doses of COVID-19 vaccination received (none, one, two or three) for each age group (20-29, 30-39, 40-49, 50-59, 60-69, 70-79, ≥80 years) and calendar day throughout the study period. Additional stratification by vaccine type was included to estimate VE for each vaccine type and relative VE (rVE) between two and three doses of each vaccine. Vaccination status was categorised according to the date of vaccination plus a 14-day lag for all doses, to allow for the delay in immune response to vaccination. Daily numbers of persons in each vaccination category were inferred from the uptake data assuming that individuals received the same vaccine for first and second dose (aligned with Hong Kong guidelines), and using aggregate data by age on vaccine switching for the third dose. The population at risk in each stratum was matched to the report date of cases, and cumulative numbers of previous SARS-CoV-2 infections within each group were removed from the population at risk at each time point. Incidence rate ratios (IRR) were estimated using a negative binomial rate model for the daily counts of cases adjusted for age group and calendar day including the logarithm of person-time as an offset term in the model to account for differing numbers at risk within each strata. VE was defined as (1-IRR)×100%.

RESULTS

A total of 486,074 persons had confirmed SARS-CoV-2 infection during the study period from December 31, 2021 to March 8, 2022. The case data were linked to the Hospital Authority dataset to determine their clinical outcomes and those with complete age and vaccination records were extracted. Of these, 5,474 persons were recorded as having mild/moderate disease between December 31, 2021 and February 15, 2022. During the entire study period from December 31, 2021 to March 8, 2022, 5,294 persons with severe/fatal disease and 4,093 with fatal disease were included (Table 1).

Up to March 8, 2022, a total of 12.7 million vaccine doses had been administered in Hong Kong. Severe disease or death occurred a median of 161 (interquartile range, IQR: 73 to 207) days after the second vaccination in those vaccinated with two doses of BNT162b2, and 127 (IQR: 51 to 162) among those who received two doses of CoronaVac. Those experiencing severe and fatal outcomes after a third dose tested positive a median of 52 (IQR: 38 to 70) days and 45 (IQR: 24 to 100) days after vaccination with BNT162b2 and CoronaVac respectively. The distribution of mild cases according to age and vaccination status were similar to the population, with severe disease and death occurring predominantly in the unvaccinated older population (Figure 2).

VE after receipt of two doses

We found two doses of CoronaVac provided no protection against mild/moderate disease across all age groups, with some protection offered by BNT162b2 in younger age groups (VE: 31.0%, 95% CI: 1.6%, 51.7%). However, both vaccines were estimated to have high effectiveness against severe disease. Limited differences in vaccine effectiveness were observed for severe outcomes in younger adults, where VE was estimated to be 95.2% (95%

CI: 92.9%, 96.8%) for BNT162b2 and 91.7% (95% CI: 87.8%, 94.4%) for CoronaVac (Table 2). The difference in VE was more pronounced for older adults, with higher effectiveness among adults >60 years who received BNT162b2 (VE: 88.2%, 95% confidence interval, CI: 84.4%, 91.1%) compared to CoronaVac (VE: 74.1%, 95% CI: 67.8%, 79.2%). When broken down further by age, we estimated that VE was 91.1% (95% CI: 85.4%, 94.6%) for BNT162b2 and 82.6% (74.2%, 88.2%) for CoronaVac in those 60-69y, reducing to 84.5% (95% CI: 75.5%, 90.2%) and 60.2% (95% CI: 43.9%, 71.8%) among those ≥80y for BNT162b2 and CoronaVac, respectively. This was also observed for the mortality endpoint, where in adults aged ≥80y two doses of BNT162b2 offered a higher level of protection against fatal disease (88.2%, 95% CI: 80.2%, 93.0%) compared to two doses of CoronaVac (66.8%, 95% CI: 51.9%, 77.0%).

We compared the two-dose schedules of both vaccines and found no significant differences between BNT162b2 and CoronaVac for mild disease in any age group. Superiority of the two-dose BNT162b2 schedule was estimated for severe/fatal disease in adults ≥60y (relative VE: 54.6%, 95% CI: 38.7%, 66.4%). This was also the case for mortality in those ≥60y (relative VE: 58.5%, 95% CI: 70.7%, 41.3%). No differences between vaccines were found against severe/fatal or fatal COVID-19 in adults 20-59y.

VE after receipt of three doses

We estimated three doses of both vaccines offered very high protection against severe disease (98.1%, 95% CI: 97.1%, 98.8%) and mortality (98.6%, 95% CI: 97.7%, 99.2%) which was sustained within all age groups (Table 2). Vaccine estimates were very similar for both vaccines against severe and fatal outcomes. Three doses of BNT162b2 was estimated to have a VE of 71.5% (95% CI: 54.5%, 82.1%) against mild/moderate disease in younger adults

while for three doses of CoronaVac the VE was estimated as 42.3% (95% CI: 11.4%, 62.4%) against the same outcome.

Relative VE of three versus two doses

We estimated the relative effect of three doses versus two doses of each vaccine type (Table 3). For mild/moderate disease we find an additional benefit of a third dose of BNT162b2 in younger (relative VE: 58.6%, 95% CI: 34.4%, 73.9%) and older (relative VE: 63.8%, 95% CI: 26.7%, 82.1%) adults who had previously received two doses of BNT162b2. A third dose of CoronaVac increased protection (relative VE: 57.0%, 95% CI: 23.4%, 75.9%) in older adults who had received two doses of CoronaVac, with no benefit observed in the younger age category. For severe/fatal disease we found an additional benefit of a third dose in adults of all ages for both vaccine types, with relative VE of 71.9% (95% CI: 25.1%, 89.5%) for three vs two doses of BNT162b2, and 96.6% (95% CI: 85.7%, 99.2%) for three vs two doses of CoronaVac among those ≥80 years. Additional protection against mortality was offered by a third dose in older adults, with no differences observed in younger adults.

DISCUSSION

We used detailed population-level data on the vaccination programme in Hong Kong since February 2021 and individual-level COVID-19 case data from December 31, 2021 to March 8, 2022 to estimate VE of one, two and three doses of BNT162b2 and CoronaVac vaccines in a largely infection-naïve population during the fifth wave of COVID-19 in Hong Kong. Two or three doses of BNT162b2 or three doses of CoronaVac provide a very high level of protection against severe disease and death in those under 80 years of age. A reduction in VE was observed among two-dose CoronaVac recipients ≥80 years. We found no effect of two doses of CoronaVac and a limited effect of BNT162b2 against mild/moderate disease, with

245 the caveat that many individuals had received their second dose several months before
246 exposure to the SARS-CoV-2 virus. Limited protection against mild/moderate disease was
247 restored with third doses for both vaccines, but we were only able to estimate VE for the
248 short period since administration of third vaccine doses, and it is unclear how long this
249 protection will last.
250
251 Although improved effectiveness of a third dose was observed against severe outcomes in
252 younger age groups, the absolute VE of two doses remains high in this age group for both
253 vaccines and the relative effects should be interpreted accordingly.[29] Our finding that three
254 doses of CoronaVac are needed for older adults to achieve high levels of protection is
255 consistent with World Health Organization recommendations for this group.[30] While there is
256 a preferential recommendation in Hong Kong for a third dose of BNT162b2 in adults who
257 received two doses of CoronaVac,[31] this did not translate to preference in the community. Of
258 all adults who had received two doses of CoronaVac and a third dose, only 26% received the
259 third dose with BNT162b2. We were unable to evaluate the comparative effectiveness of
260 heterologous vs homologous third dose schedules or durability of three dose protection in this
261 study, but evidence from our analyses that three doses of inactivated vaccine provides a high
262 level of protection against the severe spectrum of COVID-19 disease, at least in the short
263 term, is reassuring.
264
265 Almost all sequenced SARS-CoV-2 isolates during Hong Kong's fifth wave are of the
266 Omicron BA.2 lineage. Our overall findings are largely consistent with existing VE evidence
267 against this subvariant.[32–34] A study from Qatar estimated that third dose VE for BNT162b2
268 was 43.7% (95% CI: 36.5, 50.0%) in the first month and begins to decline again in the
269 following weeks, with substantially improved protection against severe outcomes (six-week

VE: 90.9%, 95% CI: 78.6%, 96.1%).[35] Similarly, a US study estimated VE of two doses of mRNA vaccines against severe Omicron disease, defined as COVID-19 requiring invasive mechanical ventilation or in-hospital death, of 79% (95% CI: 66%, 87%) a median of 265 days after the second dose; and three dose VE of 94% (95% CI: 88%, 97%), similar to our estimate of 98.1% (95% CI: 97.1%, 98.8%).[36]

Despite the overall consistency between our results and those presented in other studies, it is possible that VE, particularly against severe outcomes, has been overestimated in our study. Vaccine hesitancy in Hong Kong is highest among the elderly and in individuals with underlying health conditions.[37] In this scenario so-called 'healthy vaccinee bias', by which vaccine recipients are healthier than their unvaccinated peers, may inflate the estimates.[38] Although we have accounted for age in the current estimates, a lack of individual-level data on controls mean that this cannot be formally assessed with currently available data. However, our estimates for BNT162b2 and CoronaVac are similar to other studies using alternative designs, and we anticipate the magnitude of overestimation is unlikely to be substantial.[19,35] Even if individual-level adjustments had been possible, estimating absolute VE after vaccines have been available for some time is problematic because it is necessary to compare incidence rates in vaccinated individuals with those from unvaccinated cohorts often with few remaining persons. This is the case in younger age groups in Hong Kong, whose characteristics are likely to differ substantially from those who chose to be vaccinated earlier. This bias, inherent to observational studies, is present in much of the existing VE literature at this stage of the pandemic. To address this concern, we also estimated a relative VE of three versus two doses of each vaccine type, as these cohorts are likely to be more comparable (Table 3). We find a third dose of either vaccine provides additional protection, reiterating the public health value of a third dose for minimizing severe disease and death but also for

reducing health system congestion, public concern and indirect costs stemming from milder episodes during a COVID-19 epidemic.

We compared performance of the mRNA BNT162b2 and inactivated CoronaVac vaccines and found higher VE for BNT162b2 following one and two doses, but similar performance after three doses (Table 2). Our estimates are likely to be affected by time since vaccination, where typically more time has passed since administration of second than third doses which have only been widely available in Hong Kong since the beginning of January 2022 (Table 1). Improved effectiveness may partially reflect a recent, rather than a third, vaccine dose. This hypothesis is supported by data from an observational study in Malaysia which compared the duration of protection of the BNT162b2 and CoronaVac vaccines. They find more rapid waning of CoronaVac, in particular for mild/moderate and severe outcomes, but to a lesser extent for COVID-19 related mortality.[24] Moreover, a recent study of humoral and cellular responses among Hong Kong vaccinees over time found that neutralising antibodies against variants of concern dropped to detection limit only three months after vaccinations, along with diminishing memory T cell responses, primarily among CoronaVac recipients.[39]

Our study has a number of limitations arising from available data and the nature of the epidemic within Hong Kong. Firstly, we used census data from the correct time period to construct the source population, but any differential population movement by vaccine status over the duration of the vaccination program could affect the validity of our estimates. Furthermore, as we are estimating vaccine effectiveness in real-time, there are large amounts of missingness in clinical data, which is especially problematic when assuming a population level denominator, as the assumed number of people still at risk will be overestimated. However, this is mostly an issue for mild/moderate outcomes, as we used complete records

on COVID-19 mortality to derive estimates and we expect severe cases are fully documented. Secondly, there are some differences in testing requirements by vaccine status, particularly for those required to regularly test because of occupation. However, we expect that VE estimates against severe outcomes will be only marginally susceptible to biases related to testing requirements. Finally, in Hong Kong there was a clear preference for the BNT162b2 vaccine in younger age groups and for CoronaVac in older adults. We have addressed this confounding in estimates presented by stratifying by age categories and adjusting estimates by 10-year age categories and calendar day, however some residual confounding by age is possible in the vaccine platform-specific estimates and other factors may confound the relationship between vaccine status, type and risk of infection that cannot be accounted for in this design.

Our findings indicate that two dose schedules of both BNT162b2 and CoronaVac vaccines offer strong protection against severe disease and death, however higher levels of protection were observed among those who received two doses of BNT162b2 compared to those receiving two doses of CoronaVac, particularly in older age groups. Three recent doses of both vaccines offer very high levels of protection for older adults against severe outcomes, with no differences observed across vaccine types. It will be important to increase uptake of third vaccine doses, particularly in older adults who have so far received two doses of CoronaVac. Further investigation of the durability of protection provided by both vaccines is warranted and planned.

343 **ACKNOWLEDGMENTS**

344 The authors thank the Hong Kong Special Administrative Region Government and Hospital
345 Authority for the timely sharing of COVID-19 vaccination and case data. The authors thank
346 Julie Au for administrative support.

347

348 **FINANCIAL SUPPORT**

349 This project was supported by the China CDC-sponsored COVID-19 Vaccines Evaluation
350 Program (COVEP). BJC is supported by the Theme-based Research Scheme (project no.
351 T11-712/19-N) from the Research Grants Council from the University Grants Committee of
352 Hong Kong, and an RGC Senior Research Fellowship (grant number: HKU SRFS2021-
353 7S03).

354

355 **POTENTIAL CONFLICTS OF INTEREST:**

356 BJC reports honoraria from AstraZeneca, Fosun Pharma, GlaxoSmithKline, Moderna, Pfizer,
357 Roche and Sanofi Pasteur. JN was previously employed by and owns shares in Sanofi. The
358 authors report no other potential conflicts of interest.

359

360

REFERENCES

1. Cowling B, Ali S, Ng T, et al. Impact assessment of non-pharmaceutical interventions against coronavirus disease 2019 and influenza in Hong Kong: an observational study. *Lancet Public Health*. 2020;5(5):E297-E288.

2. The HK SAR Government. COVID-19 vaccination dashboard. Published 2022. Accessed March 17, 2022. https://www.covidvaccine.gov.hk/en/dashboard

3. HK SAR Government. Latest situation of COVID-19 (as of 16 March 2022). Published 2022. Accessed March 17, 2021. https://www.chp.gov.hk/files/pdf/local_situation_covid19_en.pdf

4. The HK SAR Government. Third dose COVID-19 vaccination arrangements for persons under certain groups. Published online November 3, 2022. Accessed March 17, 2022. https://www.info.gov.hk/gia/general/202111/03/P2021110300536.htm

5. The HK SAR Government. Further expansion of COVID-19 vaccination arrangements from January 1. Published online December 24, 2021. Accessed March 17, 2022. https://www.info.gov.hk/gia/general/202112/24/P2021122400509.htm

6. Tsang J. Coronavirus: vaccine outreach teams to visit 'all Hong Kong care facilities by Friday', at home jabs to be offered to residents with mobility issues. *South China Morning Post*. March 13, 2022.

7. news. gov.hk. Jab time interval to be shortened. news.gov.uk. Published 2022. Accessed March 18, 2022. https://www.news.gov.hk/eng/2022/03/20220304/20220304_174704_235.html

8. Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. *BMJ*. 2021;373:n1088-n1088. doi:10.1136/bmj.n1088

9. Hall VJ, Foulkes S, Saei A, et al. COVID-19 vaccine coverage in health-care workers in England and effectiveness of BNT162b2 mRNA vaccine against infection (SIREN): a prospective, multicentre, cohort study. *Lancet Lond Engl*. 2021;397(10286):1725-1735. doi:10.1016/S0140-6736(21)00790-X

10. Thompson M, Burgess J, Naleway A, et al. Interim Estimates of Vaccine Effectiveness of BNT162b2 and mRNA-1273 COVID-19 Vaccines in Preventing SARS-CoV-2 Infection Among Health Care Personnel, First Responders, an Other Essential and Frontline Workers - Eight U.S. Locations, December 2020-March 2. *MMWR Morb Mortal Wkly Rep*. 2021;70:495-500.

11. Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *N Engl J Med*. 2021;384(15):1412-1423. doi:10.1056/NEJMoa2101765

12. Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. *N Engl J Med*. 2021;385(7):585-594. doi:10.1056/NEJMoa2108891

13. Pouwels K, Pritchard E, Matthews PC, et al. Effect of Delta variant on viral burden and vaccine effectiveness against new SARS-CoV-2 infections in the UK. *Nat Med*. 2021;27:2127-2135. doi:https://doi.org/10.1038/s41591-021-01548-7

14. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med.* 2020;383(27):2603-2615. doi:10.1056/NEJMoa2034577

15. Higdon MM, Wahl B, Jones CB, et al. A systematic review of COVID-19 vaccine efficacy and effectiveness against SARS-CoV-2 infection and disease. *medRxiv*. Published online January 1, 2022:2021.09.17.21263549. doi:10.1101/2021.09.17.21263549

16. Goldberg Y, Mandel M, Bar-On YM, et al. Waning Immunity after the BNT162b2 Vaccine in Israel. *N Engl J Med.* 2021;385(24):e85-e85. doi:10.1056/NEJMoa2114228

17. Israel A, Merzon E, Schäffer AA, et al. Elapsed time since BNT162b2 vaccine and risk of SARS-CoV-2 infection: test negative design study. *BMJ*. 2021;375:e067873. doi:10.1136/bmj-2021-067873

18. Andrews N, Tessier E, Stowe J, et al. Duration of Protection against Mild and Severe Disease by Covid-19 Vaccines. *N Engl J Med.* 2022;386(4):340-350. doi:10.1056/NEJMoa2115481

19. Andrews N, Stowe J, Kirsebom F, et al. Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529) Variant. *N Engl J Med.* Published online March 2, 2022. doi:10.1056/NEJMoa2119451

20. Collie S, Champion J, Moultrie H, Bekker LG, Gray G. Effectiveness of BNT162b2 Vaccine against Omicron Variant in South Africa. *N Engl J Med.* 2022;386(5):494-496. doi:10.1056/NEJMc2119270

21. Buchan SA, Chung H, Brown KA, et al. Effectiveness of COVID-19 vaccines against Omicron or Delta symptomatic infection and severe outcomes. *medRxiv*. Published online 2022:2021.12.30.21268565. doi:10.1101/2021.12.30.21268565

22. UKHSA. *COVID-19 Vaccine Surveillance Report*.; 2022:12-13. Accessed March 18, 2022. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1057599/Vaccine_surveillance_report_-_week-8.pdf

23. Cerqueira-Silva T, Katikireddi SV, de Araujo Oliveira V, et al. Vaccine effectiveness of heterologous CoronaVac plus BNT162b2 in Brazil. *Nat Med*. Published online February 9, 2022. doi:10.1038/s41591-022-01701-w

24. Suah JL, Husin M, Keng Tok PS, et al. Waning COVID-19 Vaccine Effectiveness for BNT162b2 and CoronaVac in Malaysia: An Observational Study. *medRxiv*. Published online January 1, 2022:2022.01.15.22269326. doi:10.1101/2022.01.15.22269326

25. Suryatma A, Anasi R, Hananto M, et al. Effectiveness of the Inactivated COVID-19 Vaccine (CoronaVac) in Adult Population in Bali, Indonesia. *medRxiv*. Published online 2022:2022.02.02.22270351. doi:10.1101/2022.02.02.22270351

26. Jara A, Undurraga EA, González C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *N Engl J Med*. 2021;385(10):875-884. doi:10.1056/NEJMoa2107715

27. Haas EJ, Angulo FJ, McLaughlin JM, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. *The Lancet*. 2021;397(10287):1819-1829. doi:10.1016/S0140-6736(21)00947-8

448 28. Kong X, Yang Y, Gao J, et al. Overview of the health care system in Hong Kong and its
449 referential significance to mainland China. *J Chin Med Assoc*. 2015;78(10):569-573.
450 doi:https://doi.org/10.1016/j.jcma.2015.02.006

451 29. McMenamin ME, Bond HS, Sullivan SG, Cowling BJ. Estimation of Relative Vaccine
452 Effectiveness in Influenza: A Systematic Review of Methodology. *Epidemiology*.
453 Published online 2022.
454 https://journals.lww.com/epidem/Fulltext/9000/Estimation_of_Relative_Vaccine_Effect
455 iveness_in.98179.aspx

456 30. World Health Organisation. The Sinovac-CoronaVac COVID-19 vaccine: What you
457 need to know. who.int. Published September 2, 2021. Accessed March 21, 2022.
458 https://www.who.int/news-room/feature-stories/detail/the-sinovac-covid-19-vaccine-
459 what-you-need-to-know

460 31. Scientific Committee on Emerging and Zoonotic Diseases and Scientifc Committee on
461 the Use of COVID-19 Vaccines in Hong Kong. *Consensus Interim Recommendations*
462 *on the Use of COVID-19 Vaccines in Hong Kong*. Centre for Health Protection; 2022.
463 Accessed March 21, 2022.
464 https://www.chp.gov.hk/files/pdf/consensus_interim_recommendations_on_the_use_of_
465 covid19_vaccines_in_hong_kong_25feb.pdf

466 32. Kok KH, Wong SC, Chan WM, et al. Co-circulation of two SARS-CoV-2 variant strains
467 within imported pet hamsters in Hong Kong. *Emerg Microbes Infect*. 2022;11(1):689-
468 698. doi:10.1080/22221751.2022.2040922

33. Cheng VCC, Ip JD, Chu AWH, et al. Rapid spread of SARS-CoV-2 Omicron subvariant BA.2 in a single-source community outbreak. *Clin Infect Dis*. Published online March 10, 2022:ciac203. doi:10.1093/cid/ciac203

34. UKHSA. *COVID-19 Vaccine Surveillance Report*. UKHSA; 2022:13. Accessed March 20, 2022. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1061532/Vaccine_surveillance_report_-_week_11.pdf

35. Chemaitelly H, Ayoub HH, AlMukdad S, et al. Duration of mRNA vaccine protection against SARS-CoV-2 Omicron BA.1 and BA.2 subvariants in Qatar. *medRxiv*. Published online January 1, 2022:2022.03.13.22272308. doi:10.1101/2022.03.13.22272308

36. Tenforde M, Self W, Gaglani M, et al. Effectiveness of mRNA Vaccination in Preventing COVID-19-Associated Invasive Mechanical Ventilation and Death - United States, March 2021-January 2022. *MMWR Morb Mortal Wkly Rep*. 2022;ePub: 18 March 2022. doi:http://dx.doi.org/10.15585/mmwr.mm7112e1

37. Xiao J, Cheung J, Wu P, Ni M, Cowling B, Liao Q. Temporal changes in factors associated with COVID-19 vaccine hesitancy and uptake among adults in Hong Kong: Serial cross-sectional surveys. *Lancet Reg Health - West Pac*. 2022;(In press).

38. Remschmidt C, Wichmann O, Harder T. Frequency and impact of confounding by indication and healthy vaccinee bias in observational studies assessing influenza vaccine effectiveness: a systematic review. *BMC Infect Dis*. 2015;15(1):429. doi:10.1186/s12879-015-1154-y

491 39. Peng Q, Zhou R, Wang Y, et al. Waning immune responses against SARS-CoV-2
492 variants of concern among vaccinees in Hong Kong. *eBioMedicine*. 2022;77.
493 doi:10.1016/j.ebiom.2022.103904

494

495

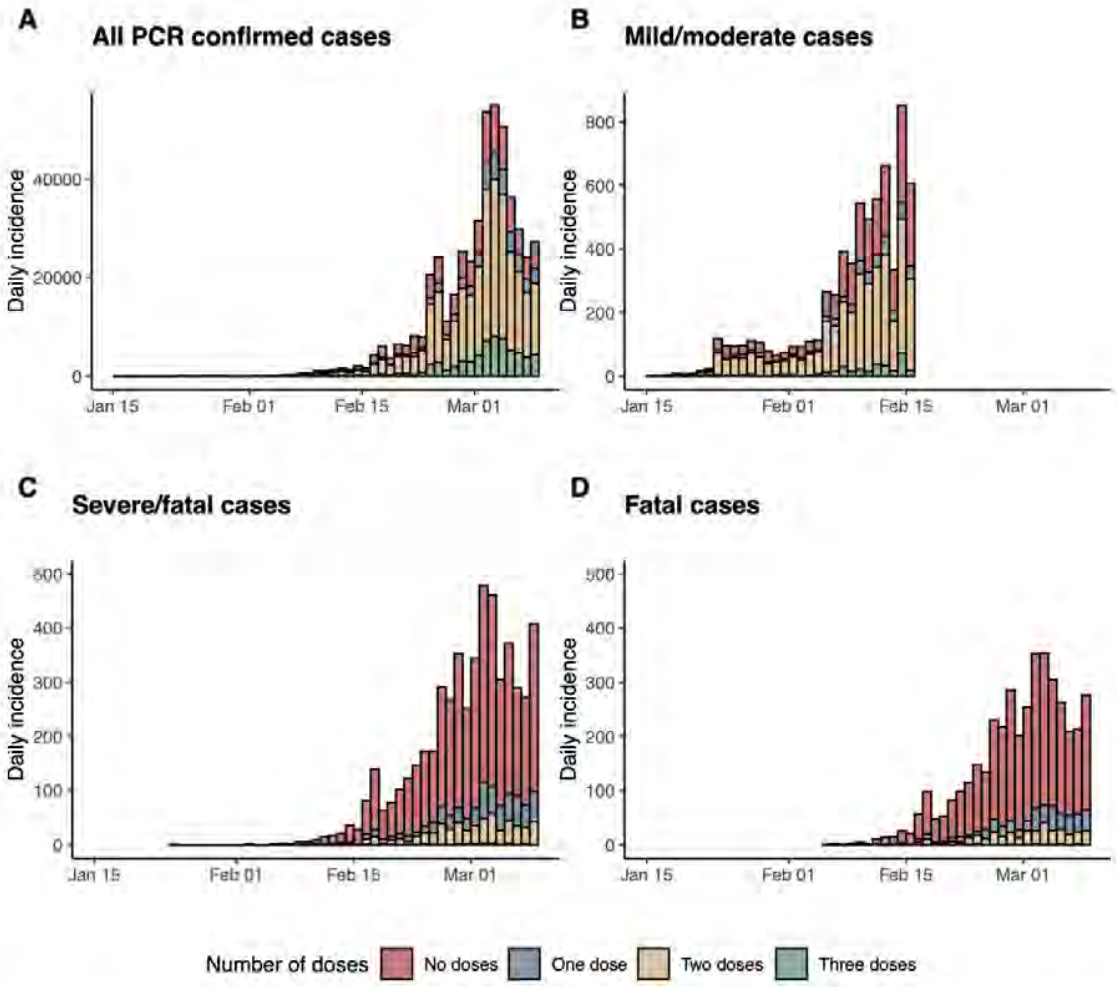


Figure 1. Daily incidence of (A) all PCR confirmed COVID-19 cases (B) mild/moderate cases in the early part of the fifth wave prior to 15 February 2022, (C) severe/fatal cases, and (D) deaths throughout the fifth wave in Hong Kong by vaccination status, where severe disease is defined as having ever been listed as 'Serious' or 'Critical' or 'Fatal' by the Hospital Authority during hospitalisation for COVID-19. Vaccination status was categorised according to the number of doses received plus a 14-day lag for all doses, to allow for the immune response to vaccination. The drop in mild/moderate cases on 4 March was due to a very small number of cases being reported as having been admitted to hospital or isolation facilities on that day. Mild cases were only included up until 15 February 2022 to account for change in admission criteria.

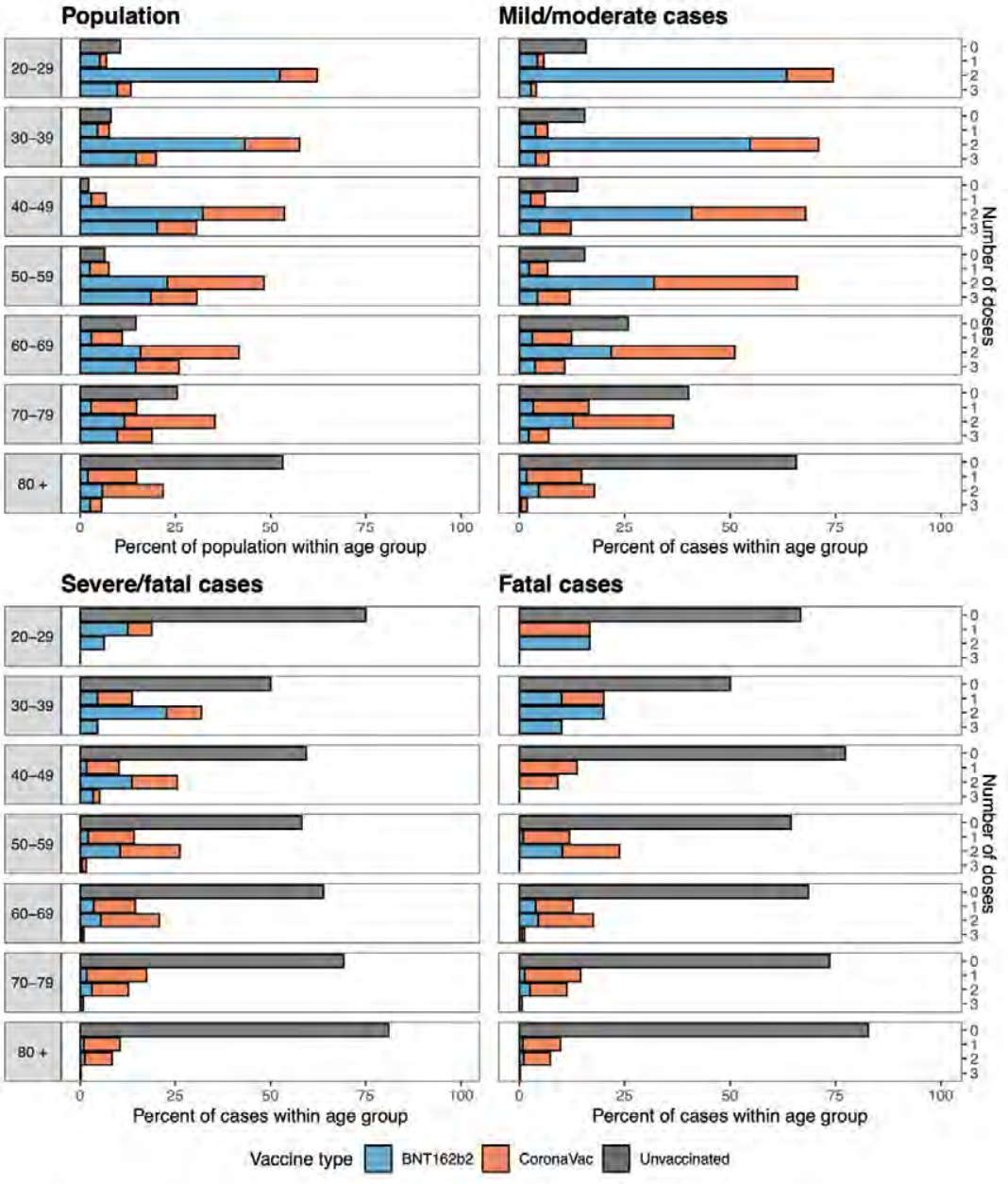


Figure 2. Vaccine status of population and those experiencing mild/moderate, severe/fatal and fatal COVID-19 as at 8 March 2022 as a percent of the population within a given age group shown by vaccine type and number of doses.

Table 1. Descriptive characteristics of confirmed COVID-19 cases in Hong Kong classified as having mild, severe or fatal disease between 31 December 2021 and 8 March 2022.

		Mild/moderate disease (N= 5474)	Severe/fatal disease (N=5294)	Fatal disease (N=4093)
Age				
20-49 years		3144	101	39
50-69 years		1602	784	488
≥70 years		728	4408	3566
Sex				
Male		2337	3245	2528
Female		3137	2049	1565
Vaccination status[a]				
No doses		1300	4064	3277
One dose				
	BNT162b2	151	73	44
	CoronaVac	226	532	374
Two doses				
	BNT162b2	2139	130	74
	CoronaVac	1271	434	287
Three doses				
	BNT162b2	126	12	7
	CoronaVac	210	14	7
Median (25th, 75th percentile) of days between last vaccine dose and positive SARS-CoV-2 test result[b]				
One dose				
	BNT162b2	27 (22, 35)	21 (18, 32)	21 (18, 32)
	CoronaVac	29 (21, 35)	24 (17, 38)	24 (17, 39)
Two doses				
	BNT162b2	182 (151, 217)	161 (73, 207)	171 (91, 213)
	CoronaVac	179 (146, 209)	127 (51, 162)	125 (51, 157)
Three doses				
	BNT162b2	31 (20, 49)	52 (38, 70)	68 (49, 77)
	CoronaVac	39 (25, 66)	45 (24, 100)	64 (30, 100)

[a]Number of doses plus 14-day lag
[b]Median time since vaccination among those where 14 days has passed since latest dose

Table 2. Vaccine effectiveness by dose (one, two, three) and vaccine type (CoronaVac, BNT162b2) in all ages and within age categories (mild/moderate: 20-59, ≥60; severe/fatal, fatal: 20-59, 60-69, 70-79, ≥80 years) against COVID-19 related mild/moderate disease, severe/fatal disease and death.

	One dose		Two doses		Three doses	
	BNT162b2	**CoronaVac**	**BNT162b2**	**CoronaVac**	**BNT162b2**	**CoronaVac**
Mild/moderate disease						
20-59 years	37.4	2.1	31.0	17.9	71.5	42.3
	(0.7, 60.6)	(-53.3, 37.5)	(1.6, 51.7)	(-18.0, 42.9)	(54.5, 82.1)	(11.4, 62.4)
≥60 years	None[a]	None[a]	None[a]	None[a]	71.6	50.7
					(43.5, 85.7)	(12.9, 72.1)
Severe/fatal disease						
20-59 years	85.0	60.9	95.2	91.7	98.5	98.5
	(69.1, 92.7)	(40.6, 74.3)	(92.9, 96.8)	(87.8, 94.4)	(95.9, 99.4)	(95.2, 99.5)
60-69 years	59.9	55.1	91.1	82.6	99.2	98.5
	(29.3, 77.3)	(30.9, 70.9)	(85.4, 94.6)	(74.2, 88.2)	(96.7, 99.8)	(95.3, 99.6)
70-79 years	71.5	33.9	89.4	80.8	99.5	96.7
	(48.9, 84.1)	(8.1, 52.5)	(83.0, 93.3)	(72.8, 86.5)	(96.0, 99.9)	(92.3, 98.6)
≥80 years	65.0	35.0	84.5	60.2	95.7	98.6
	(42.2, 78.8)	(8.8, 53.7)	(75.5, 90.2)	(43.9, 71.8)	(89.0, 98.3)	(94.3, 99.7)
Mortality						
20-59 years	93.7	65.4	96.4	94.0	99.4	-[b]
	(74.2, 98.5)	(38.6, 79.4)	(93.6, 98.0)	(89.6, 96.5)	(95.6, 99.9)	-
60-69 years	63.3	70.2	93.7	87.6	98.9	98.7
	(30.7, 80.5)	(51.3, 81.7)	(88.6, 96.5)	(80.9, 91.9)	(95.3, 99.7)	(94.4, 99.7)
70-79 years	81.3	48.9	92.2	84.4	-[b]	97.2
	(60.6, 91.1)	(28.1, 63.7)	(86.5, 95.5)	(77.5, 89.2)	-	(92.3, 99.0)
≥80 years	71.8	40.5	88.2	66.8	96.0	99.2
	(50.6, 83.9)	(14.9, 58.4)	(80.2, 93.0)	(51.9, 77.0)	(88.8, 98.6)	(94.3, 99.9)

526

527 [a] No evidence of protection based on a negative or very small positive point estimate and wide confidence

528 intervals.

529 [b] Insufficient outcomes to estimate

530

531

532

Table 3. Relative vaccine effectiveness of a three versus two dose BNT162b2 schedule and a three versus two dose CoronaVac schedule against mild disease, severe disease and mortality as defined by the Hospital Authority.

	Relative VE of three doses vs two doses of same vaccine technology (%)	
	CoronaVac	**BNT162b2**
Mild/moderate disease		
20-59 years	29.7 (-7.7, 54.1)	58.6 (34.4, 73.9)
≥60 years	57.0 (23.4, 75.9)	63.8 (26.7, 82.1)
Severe/fatal disease		
20-59 years	81.8 (40.6, 94.4)	68.3 (9.8, 88.9)
60-69 years	91.7 (72.5, 97.5)	91.1 (61.2, 98.0)
70-79 years	83.0 (58.8, 93.0)	94.9 (61.4, 99.3)
≥80 years	96.6 (85.7, 99.2)	71.9 (25.1, 89.5)
Mortality		
20-59 years	-[a]	83.1 (-28.6, 97.8)
60-69 years	89.2 (53.9, 97.4)	82.2 (20.0, 96.0)
70-79 years	82.4 (49.4, 93.8)	-[a]
≥80 years	97.7 (82.8, 99.7)	66.2 (-1.3, 88.7)

[a] Insufficient outcomes to estimate

5.8. Estudo chileno com mais de dez milhões de pessoas mostra que efetividade da CoronaVac é superior a 86%, inclusive entre idosos

Um artigo publicado no The New England Journal of Medicine mostrou mais uma vez que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, é efetiva (ou seja, tem eficácia comprovada no "mundo real" e não somente em um estudo controlado de ensaios clínicos) contra casos de Covid-19 e variantes do SARS-CoV-2, inclusive entre as pessoas com mais de 60 anos.

A pesquisa foi realizada no Chile e mostrou que a proteção da CoronaVac foi de 65,9% contra infecções por Covid-19, de 87,5% contra hospitalizações, de 90,3% contra internações em Unidades de Terapia Intensiva (UTI) e de 86,3% contra mortes. Para o grupo totalmente vacinado acima de 60 anos, a efetividade da vacina foi de 66,6% para a proteção contra infecções, de 85,3% contra hospitalizações, de 89,2% contra internações na UTI e de 86,5% para a prevenção de morte relacionada à doença.

A análise foi feita entre fevereiro e maio de 2021 com cerca de 10,2 milhões de pessoas. O estudo de coorte (estudo observacional que acompanha indivíduos ao longo de um período para determinar características e evolução do grupo) contou com participantes acima dos 16 anos cadastrados no Fundo Nacional de Saúde (FONASA), programa nacional de saúde chileno, que cobre cerca de 80% da população.

Os participantes foram divididos em três grupos: não vacinados, vacinados com apenas uma dose e totalmente vacinados. Os testes para detecção da Covid-19 foram exames RT-PCR (98,1%) e testes rápidos de antígeno (1,9%). Durante o período da análise, as UTIs no Chile operavam com 93,5% da sua capacidade.

O país andino tem as taxas mais elevadas de realização de testes para detecção da Covid-19 na América Latina e um sistema padronizado de informação pública para estatísticas vitais ao estudo. O Ministério da Saúde do país utilizou 13,98 milhões de doses da vacina CoronaVac desde o começo da campanha de vacinação, em fevereiro de 2021.

Outro estudo de efetividade da CoronaVac foi realizado pelo Butantan no município paulista de Serrana. O chamado Projeto S vacinou quase toda a população adulta do município (28 mil pessoas) entre fevereiro e abril de 2021 e concluiu que o imunizante causou uma redução de 80% no número de casos sintomáticos de Covid-19, de 86% nas internações e de 95% nos óbitos.

Publicado em: 2/09/2021

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 SEPTEMBER 2, 2021 VOL. 385 NO. 10

Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile

Alejandro Jara, Ph.D., Eduardo A. Undurraga, Ph.D., Cecilia González, M.D., Fabio Paredes, M.Sc., Tomás Fontecilla, M.Sc., Gonzalo Jara, B.S.E., Alejandra Pizarro, M.D., Johanna Acevedo, M.S., Katherinne Leo, B.S.E., Francisco Leon, M.B.A., Carlos Sans, B.S.E., Paulina Leighton, B.S.E., Pamela Suárez, B.S.E., Heriberto García-Escorza, M.S., and Rafael Araos, M.D.

ABSTRACT

BACKGROUND

Mass vaccination campaigns to prevent coronavirus disease 2019 (Covid-19) are occurring in many countries; estimates of vaccine effectiveness are urgently needed to support decision making. A countrywide mass vaccination campaign with the use of an inactivated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine (CoronaVac) was conducted in Chile starting on February 2, 2021.

METHODS

We used a prospective national cohort, including participants 16 years of age or older who were affiliated with the public national health care system, to assess the effectiveness of the inactivated SARS-CoV-2 vaccine with regard to preventing Covid-19 and related hospitalization, admission to the intensive care unit (ICU), and death. We estimated hazard ratios using the extension of the Cox proportional-hazards model, accounting for time-varying vaccination status. We estimated the change in the hazard ratio associated with partial immunization (≥14 days after receipt of the first dose and before receipt of the second dose) and full immunization (≥14 days after receipt of the second dose). Vaccine effectiveness was estimated with adjustment for individual demographic and clinical characteristics.

RESULTS

The study was conducted from February 2 through May 1, 2021, and the cohort included approximately 10.2 million persons. Among persons who were fully immunized, the adjusted vaccine effectiveness was 65.9% (95% confidence interval [CI], 65.2 to 66.6) for the prevention of Covid-19 and 87.5% (95% CI, 86.7 to 88.2) for the prevention of hospitalization, 90.3% (95% CI, 89.1 to 91.4) for the prevention of ICU admission, and 86.3% (95% CI, 84.5 to 87.9) for the prevention of Covid-19–related death.

CONCLUSIONS

Our results suggest that the inactivated SARS-CoV-2 vaccine effectively prevented Covid-19, including severe disease and death, a finding that is consistent with results of phase 2 trials of the vaccine. (Funded by Agencia Nacional de Investigación y Desarrollo and others.)

From the Ministry of Health (A.J., C.G., F.P., T.F., G.J., A.P., J.A., K.L., F.L., C.S., P.L., P.S., H.G.-E., R.A.), Facultad de Matemáticas (A.J.) and Escuela de Gobierno (E.A.U.), Pontificia Universidad Católica de Chile, Millennium Nucleus Center for the Discovery of Structures in Complex Data (A.J.), Millennium Initiative for Collaborative Research in Bacterial Resistance (E.A.U., R.A.), the Research Center for Integrated Disaster Risk Management (E.A.U.), Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina, Clínica Alemana Universidad del Desarrollo (R.A.), and the Advanced Center for Chronic Diseases (R.A.) — all in Santiago, Chile; and the CIFAR Azrieli Global Scholars Program, CIFAR, Toronto (E.A.U.). Address reprint requests to Dr. Araos at Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina, Clínica Alemana Universidad del Desarrollo, Av. Las Condes 12461, Las Condes 7590943, Chile, or at rafaelaraos@udd.cl.

Drs. Jara, Undurraga, and Araos contributed equally to this article.

This article was published on July 7, 2021, and updated on July 13, 2021, at NEJM.org.

N Engl J Med 2021;385:875-84.
DOI: 10.1056/NEJMoa2107715

THE CORONAVIRUS DISEASE 2019 (COVID-19) pandemic has imposed an enormous disease burden worldwide, with more than 159 million cases and approximately 3.3 million deaths reported as of May 10, 2021.[1] Covid-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and the severity ranges from mild symptoms to life-threatening disease.[2] Older age and underlying conditions substantially increase the case fatality rate.[3,4] Nonpharmaceutical interventions, such as social distancing, face masks, and contact tracing, have so far been the mainstay of health policy strategies to reduce viral spread and limit demands on health care.[5,6] New Covid-19 vaccines are beginning to change this situation. On December 2, 2020, the first vaccine tested in a large, randomized clinical trial was approved in the United Kingdom,[7,8] although some countries began vaccinations before clinical results were available. Several effective vaccines against Covid-19 have been developed and approved in record time,[8-12] and numerous new vaccines are in the final stages of clinical trials.[13]

Mass vaccination campaigns to prevent Covid-19 are now occurring in many countries.[14] Preliminary results of the effectiveness of other Covid-19 vaccines across different populations have been published, including studies at the national level in Israel[15] and Scotland[16] and studies involving essential frontline workers at specific locations in the United States.[17-19] Estimates of vaccine effectiveness in the prevention of Covid-19 are essential because they reflect real-world challenges, such as logistics, cold chains, vaccination schedules, and follow-up, and also involve more diverse populations than those selected in randomized clinical trials, such as older or immunocompromised persons or those with coexisting conditions. Despite being the standard for assessing vaccine efficacy, phase 3 clinical trials have some limitations, such as restrictive inclusion criteria and implementation under strict experimental conditions that may not resemble a mass vaccination rollout.[20] Thus, large observational studies to estimate the effectiveness of new vaccines in real-world settings are an essential complement to randomized, controlled trials.[21]

Existing vaccine-effectiveness estimates have focused on the BNT162b2 messenger RNA (mRNA) vaccine (Pfizer–BioNTech), the ChAdOx1 nCoV-19 vaccine (Oxford–AstraZeneca), and the mRNA-1273 vaccine (Moderna).[15-19] Several countries are conducting vaccination campaigns with the use of an inactivated SARS-CoV-2 vaccine (CoronaVac) amid a record surge of Covid-19 cases worldwide.[1,13] A total of 22 primarily low- and middle-income countries have approved the CoronaVac vaccine for emergency use. Despite its global importance, limited evidence is available on the efficacy or effectiveness of this vaccine.

Phase 1–2 trials of the CoronaVac vaccine[22] were carried out in China among participants 18 to 59 years of age[23] and in participants 60 years of age or older.[24] The findings suggested that the vaccine was safe and immunogenic in most patients 14 days after receipt of the second dose. Phase 3 clinical trials are taking place in Brazil, Chile, Indonesia, and Turkey (ClinicalTrials .gov numbers, NCT04456595, NCT04651790, NCT04508075, and NCT04582344, respectively). Efficacy results from these trials have not yet been published, but reported efficacy estimates from the manufacturers with regard to mild Covid-19 have varied substantially among the sites: 50.7% (95% confidence interval [CI], 35.6 to 62.2) in Brazil, 65.3% in Indonesia, and 83.5% (95% CI, 65.4 to 92.1) in Turkey.[25-28] In addition, preliminary estimates from an observational study involving vaccinated health care workers (from a preprint server) suggested that at least one dose of the CoronaVac vaccine was 49.6% (95% CI, 11.3 to 71.4) effective against Covid-19 in Manaus, Brazil, a location where the P.1 (or gamma) variant, which is considered to be a variant of concern by the Centers for Disease Control and Prevention,[29] is predominant (occurred in approximately 75% of the test results).[30] No estimates of the effectiveness of the CoronaVac vaccine with regard to preventing Covid-19 in the general population or in persons who have received full vaccination are publicly available.

On February 2, 2021, Chile began a mass vaccination campaign with the CoronaVac vaccine (Section S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).[31] The Public Health Institute of Chile approved the CoronaVac vaccine for emergency use on January 20, 2021; the vaccine is to be administered in a two-dose schedule, with doses separated by 28 days. The vaccination campaign prioritized older adults, beginning at 90 years of age or older; frontline health care workers; and persons with underlying conditions. The government relied on the existing health care infrastructure to roll the vaccines out to the eligible

population where they lived. Vaccination rollout was organized by means of a publicly available national schedule that assigned specific dates to eligible groups. Eligible persons needed to show up at the nearest vaccination site with their identification; they did not need to make an appointment (Figs. S3 and S4). A national immunization registry keeps track of the vaccination schedules. As of May 10, 2021, the Ministry of Health has administered 13.98 million doses of the CoronaVac vaccine (7.62 million first doses and 6.36 million second doses).[32] Vaccine introduction and scale-up of the campaign occurred during a period with the highest incidence rates of Covid-19 since the beginning of the pandemic in Chile.

We used a rich administrative observational data set to provide estimates of the effectiveness of the CoronaVac vaccine in preventing Covid-19 and related hospitalization, admission to the intensive care unit (ICU), and death in the Chilean population. We estimated the effectiveness of the administration of one vaccine dose and of two doses (the complete schedule), with adjustment for relevant demographic and clinical confounders of the association between vaccination and Covid-19 outcomes. We conducted robustness checks to test whether vaccine effectiveness would be affected by differences in health care access between the vaccinated and unvaccinated groups, and we provide vaccine-effectiveness estimates among persons 16 to 59 years of age and among those 60 years of age or older.

METHODS

STUDY POPULATION AND DESIGN

We used a prospective observational cohort at the national level. The study cohort included participants 16 years of age or older who were affiliated with Fondo Nacional de Salud (FONASA), the national public health insurance program, which includes approximately 80% of the Chilean population. A detailed description of the vaccination campaign is provided in the Supplementary Appendix. Eligibility criteria included an age of 16 years or more, affiliation with FONASA, and receipt of at least one dose of the CoronaVac vaccine between February 2 and May 1, 2021, or no receipt of any Covid-19 vaccination. We excluded participants with a probable or confirmed SARS-CoV-2 infection, as assessed by reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay or antigen testing, on or before February 2, 2021, and persons who had received at least one dose of the BNT162b2 vaccine. We did not focus on the effectiveness of the BNT162b2 vaccine because these estimates have been provided elsewhere.[15,17] We focused on the results regarding the CoronaVac vaccine because they are the mainstay of the vaccination strategy in Chile. However, we provide estimates of the effectiveness of the BNT162b2 vaccine in the Supplementary Appendix as a validation of the procedures used here.

All persons 16 years of age or older are eligible to receive the vaccine, according to the national vaccination schedule. We classified participants into three groups: those who were not vaccinated, those who were partially immunized (≥14 days after receipt of the first vaccine dose and before receipt of the second dose), and those who were fully immunized (≥14 days after receipt of the second dose).

The study team was entirely responsible for the design of the study and for the collection and analysis of the data. The authors vouch for the accuracy and completeness of the data. The first, second, and last authors wrote the first draft of the manuscript.

OUTCOMES AND COVARIATES

We estimated vaccine effectiveness using four primary outcomes: laboratory-confirmed Covid-19, hospitalization for Covid-19, admission to the ICU for Covid-19, and Covid-19–related death. For all the outcomes, we considered the time from the beginning of follow-up (February 2, 2021) to the onset of symptoms as the end point. Vaccine-effectiveness estimates regarding Covid-19 cases included the more severe outcomes. All suspected cases of Covid-19 in Chile are notified to health authorities by means of an online platform and are confirmed by laboratory testing. In our study, cases of Covid-19 and related deaths were those in persons with laboratory-confirmed infection, which corresponds to code U07.1 in the *International Classification of Diseases, 10th Revision*.

We controlled for several patient characteristics that could confound the association between vaccination and outcomes, including age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19. These conditions included chronic kidney disease, diabetes, cardiovascular disease, stroke, chronic obstructive pulmonary disease, hematologic dis-

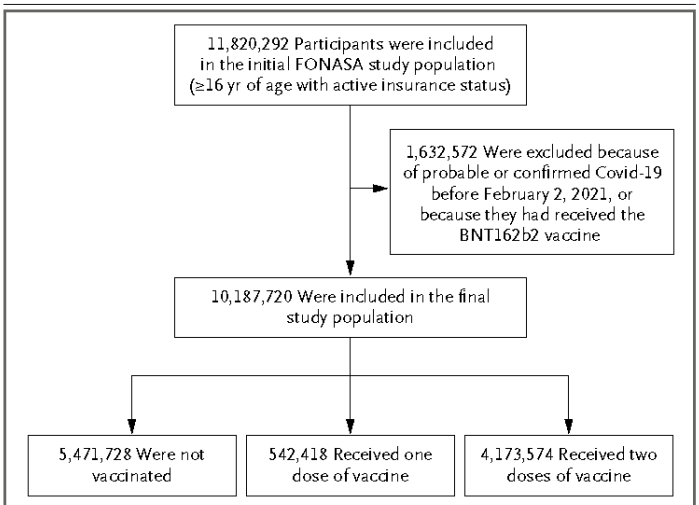


Figure 1. Study Participants and Cohort Eligibility.

Participants were at least 16 years of age, were affiliated with Fondo Nacional de Salud (FONASA; the national public health care system in Chile), and either had received at least one dose of the CoronaVac vaccine between February 2 and May 1, 2021, or had not received any vaccination. We excluded persons who had probable or confirmed coronavirus disease 2019 (Covid-19) according to reverse-transcriptase–polymerase-chain-reaction assay for severe acute respiratory syndrome coronavirus 2 and all persons who had been immunized with the BNT162b2 vaccine.

ease, autoimmune disease, human immunodeficiency virus infection, and Alzheimer's disease and other dementias.[4,33-35]

STATISTICAL ANALYSIS

Our analysis was broadly based on the analytic methods of Thompson et al.[17] for estimating vaccine effectiveness in the United States. We determined vaccine effectiveness by estimating the hazard ratio between the vaccinated and unvaccinated groups. On the basis of the observed information regarding the time to symptom onset from February 2, 2021, we estimated hazard ratios using the extension of the Cox proportional-hazards model, which allowed us to account for a time-varying vaccination status of the persons in the study. We evaluated the robustness of the model assumptions by fitting a stratified version of the extended Cox proportional-hazards model using the available predictors. Inference was based on a partial likelihood approach (Section S2).[17] We estimated the change in the hazard associated with partial immunization and full immunization, and both time-to-event analyses were performed separately. Because the immunity status induced by the CoronaVac vaccine is unknown during the 13 days between vaccine administration and partial or full immunization, those periods were excluded from the at-risk person-time in our analyses.[17]

We estimated the vaccine effectiveness as 1 minus the corresponding hazard ratio, obtained from a model including the previously described covariates, which was expressed as a percentage. We also provide the results with adjustment for the effect of sex and age only. To evaluate whether our effectiveness results were affected by potentially different access to health care between vaccinated persons and unvaccinated persons and according to the age distribution, we performed subgroup analyses involving the subgroup of persons with access to RT-PCR or antigen testing for SARS-CoV-2 and subgroups of persons 60 years of age or older and persons 16 to 59 years of age. Statistical analyses were conducted with the use of the survival package of R software, version 4.0.5.[36,37]

RESULTS

STUDY POPULATION AND VACCINATION ROLLOUT

Figure 1 shows the flow diagram of the study cohort. Of the 11,820,292 persons 16 years of age or older who were affiliated with FONASA, 10,187,720 were eligible for inclusion in the study. Table 1 shows the descriptive statistics for the approximately 10.2 million participants included in the study cohort. There were significant differences according to geographic region, sex, age, income group, nationality, and presence of underlying medical conditions, both in the incidence of Covid-19 and according to vaccination status (unvaccinated, vaccinated with only one dose, or vaccinated with two doses). Laboratory confirmation of infection was by RT-PCR assay in 98.1% of the cases and by antigen testing in 1.9%. Figure 2A shows the rapid rollout of the vaccination campaign, which started on February 2, 2021. Details of the vaccination campaign are provided in Section S1 and Figures S5 through S8. Figure 2B shows the crude cumulative incidence of Covid-19 during the study period among persons who had received one or two doses of vaccine or were unvaccinated.

VACCINE EFFECTIVENESS

There were approximately 615 million person-days in the unvaccinated group, 70 million person-days in the partially immunized group, and 92 million

Table 1. Characteristics of the Study Cohort, Overall and Those with Laboratory-Confirmed Covid-19, According to Vaccination Status.*

Characteristic	Cohort Participants		Persons with Covid-19		P Value	Unvaccinated Persons		Persons Vaccinated with One Dose		Persons Vaccinated with Two Doses		P Value
	no.	%	*no.*	%		*no.*	%	*no.*	%	*no.*	%	
Total	10,187,720	100	248,645	2.4	—	5,471,728	53.7	542,418	5.3	4,173,574	41.0	—
Sex												<0.001
Female	5,469,202	54.0	135,311	2.5	<0.001	2,775,436	50.8	272,044	5.0	2,421,722	44.3	
Male	4,718,518	46.0	113,334	2.4		2,696,292	57.1	270,374	5.7	1,751,852	37.1	
Age group												<0.001
16–19 yr	708,676	7.0	14,871	2.1	<0.001	670,451	94.6	8,192	1.2	30,033	4.2	
20–29 yr	2,017,676	20.0	59,645	3.0		1,655,595	82.1	55,854	2.8	306,227	15.2	
30–39 yr	1,867,491	18.0	54,480	2.9		1,446,544	77.5	59,166	3.1	361,781	19.4	
40–49 yr	1,423,770	14.0	39,993	2.8		851,622	59.8	165,487	11.6	406,661	28.6	
50–59 yr	1,457,564	14.0	37,539	2.6		434,694	29.8	184,268	12.6	838,602	57.5	
60–69 yr	1,365,940	13.0	23,669	1.7		221,738	16.2	41,693	3.1	1,102,509	80.7	
70–79 yr	870,082	8.5	11,778	1.4		111,592	12.8	16,412	1.9	742,078	85.3	
≥80 yr	476,521	4.7	6,670	1.4		79,492	16.7	11,346	2.4	385,683	80.9	
No. of coexisting conditions†												<0.001
0	6,880,426	68.0	168,401	2.4	0.04	4,447,684	64.6	394,030	5.7	2,038,712	29.6	
≥1	3,307,294	32.0	80,244	2.4		1,024,044	31.0	148,388	4.5	2,134,862	64.6	<0.001
Nationality												
Chilean	9,497,058	93.2	233,572	2.5	<0.001	4,913,208	51.7	513,604	5.4	4,070,246	42.9	
Non-Chilean	690,662	6.8	15,073	2.2		558,520	80.9	28,814	4.2	103,328		

* The study cohort included eligible persons who were affiliated with Fondo Nacional de Salud, the national public health insurance program, which collects, manages, and distributes funds for the public health care system in Chile. The model also included individual-level income and location (16 regions). Additional details are provided in Table S1. Covid-19 denotes coronavirus disease 2019.

† Coexisting conditions included chronic kidney disease, diabetes, cardiovascular disease (hypertension or myocardial infarction), stroke, chronic obstructive pulmonary disease, hematologic disease (lymphoma, leukemia, or myeloma), autoimmune disease (rheumatoid arthritis, juvenile idiopathic arthritis, or systemic lupus erythematosus), human immunodeficiency virus infection, and Alzheimer's disease and other dementias.

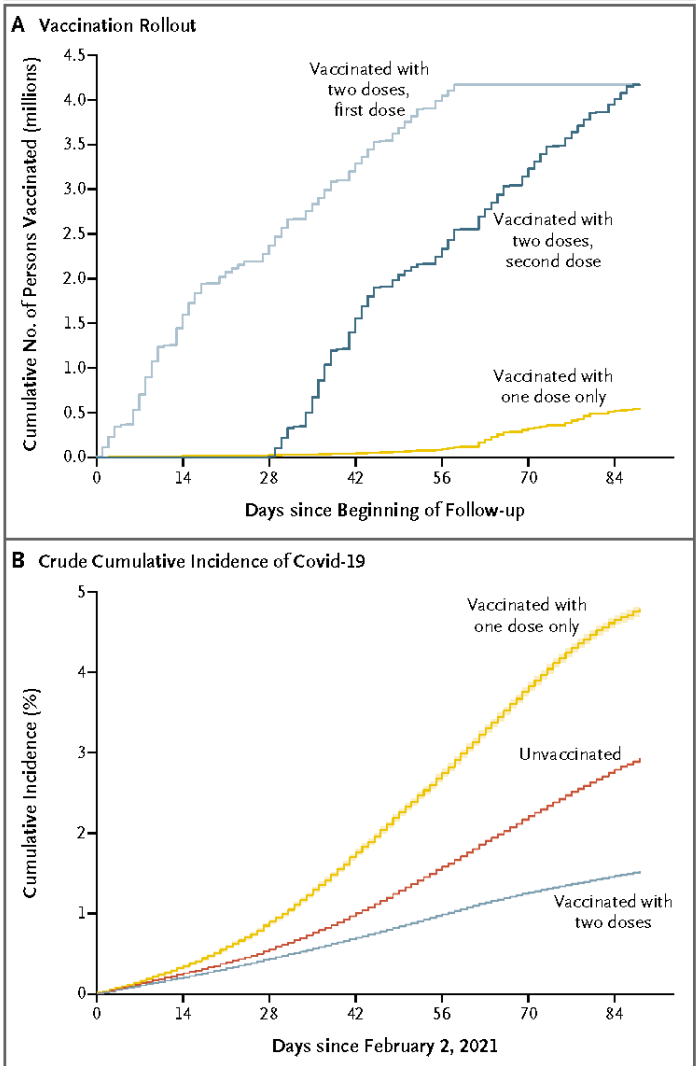


Figure 2. Vaccination Rollout and Crude Cumulative Incidence of Covid-19 in the Study Cohort.

Panel A shows the pace and coverage of the vaccination program among persons who received both doses of vaccine (first and second doses shown separately) or only one dose during the study period (February 2 through May 1, 2021). Panel B shows the crude cumulative incidence of Covid-19 during the study period among unvaccinated persons, among persons who had received only one dose of vaccine, and among persons who had received both doses of vaccine. The relatively high cumulative incidence of Covid-19 in the one-dose group should be interpreted with caution. As shown in Panel A, this group initiated vaccination approximately 40 days after the beginning of the vaccination campaign on February 2, 2021. Therefore, the incidence curve includes all cases that occurred from before vaccination up to 13 days after receipt of the first dose. Shading on the lines indicates 95% confidence intervals.

person-days in the fully immunized group during the study period (Table 2). We documented 218,784 cases of Covid-19, as well as 22,866 hospitalizations, 7873 ICU admissions, and 4042 deaths.

We estimated that the vaccine effectiveness among partially immunized persons (14 to 28 days after receipt of the first dose) was 15.5% (95% CI, 14.2 to 16.8) for the prevention of Covid-19 and 37.4% (95% CI, 34.9 to 39.9) for the prevention of hospitalization, 44.7% (95% CI, 40.8 to 48.3) for the prevention of admission to the ICU, and 45.7% (95% CI, 40.9 to 50.2) for the prevention of Covid-19–related death. In the fully immunized group, the estimated adjusted vaccine effectiveness was 65.9% (95% CI, 65.2 to 66.6) for the prevention of Covid-19 and 87.5% (95% CI, 86.7 to 88.2) for the prevention of hospitalization, 90.3% (95% CI, 89.1 to 91.4) for the prevention of ICU admission, and 86.3% (95% CI, 84.5 to 87.9) for the prevention of Covid-19–related death (Table 2). The vaccine-effectiveness estimates in the stratified model were consistent with these results.

We estimated that the adjusted vaccine effectiveness in the subgroup of fully immunized persons 60 years of age or older was 66.6% (95% CI, 65.4 to 67.8) for the prevention of Covid-19 and 85.3% (95% CI, 84.3 to 86.3) for the prevention of hospitalization, 89.2% (95% CI, 87.6 to 90.6) for the prevention of ICU admission, and 86.5% (95% CI, 84.6 to 88.1) for the prevention of Covid-19–related death (Table 3). Vaccine-effectiveness estimates among persons 16 to 59 years of age are provided in Table S3.

To address a potential concern that the observed vaccine effectiveness may have been driven by health care access, we conducted an analysis in the subgroup of persons who had undergone testing with an RT-PCR assay (98.1%) or antigen test (1.9%) during the analysis period. The results, conditional on whether testing was performed, showed larger effects for vaccination than when we included the complete cohort. Among fully immunized persons in this subgroup, the adjusted vaccine effectiveness was 72.9% (95% CI, 72.3 to 73.4) for the prevention of Covid-19 and 89.2% (95% CI, 88.5 to 89.8) for the prevention of hospitalization, 91.6% (95% CI, 90.5 to 92.5) for the prevention of ICU admission, and 87.8% (95% CI, 86.2 to 89.2) for the prevention of Covid-19–related death (Table S4).

DISCUSSION

We provide estimates of the effectiveness of administration of the CoronaVac vaccine in a countrywide mass vaccination campaign for the prevention of laboratory-confirmed Covid-19 and related hospitalization, admission to the ICU, and

Table 2. Effectiveness of CoronaVac Vaccine in Preventing Covid-19 Outcomes in Overall Study Cohort, According to Immunization Status.*

Outcome and Immunization Status	Study Cohort	Persons with Covid-19		Vaccine Effectiveness (95% CI)		
	No. of Person-Days	No. of Persons	Incidence Rate	Analysis Adjusted for Sex and Age	Analysis Adjusted for All Covariates†	Stratified Analysis‡
			no. of events/ 1000 person-days		*percent*	
Covid-19						
Unvaccinated	614,868,240	185,633	0.3019	—	—	—
Partially immunized	69,788,352	20,865	0.2990	8.0 (6.5–9.4)	15.5 (14.2–16.8)	17.2 (15.8–18.6)
Fully immunized	91,671,797	12,286	0.1340	61.2 (60.3–62.0)	65.9 (65.2–66.6)	63.7 (62.8–64.6)
Hospitalization						
Unvaccinated	620,894,706	18,034	0.0290	—	—	—
Partially immunized	70,690,796	3,370	0.0477	31.4 (28.6–34.0)	37.4 (34.9–39.9)	40.3 (37.6–42.8)
Fully immunized	92,445,333	1,462	0.0158	86.0 (85.1–86.8)	87.5 (86.7–88.2)	86.5 (85.6–87.4)
Admission to ICU						
Unvaccinated	621,226,431	6,359	0.0102	—	—	—
Partially immunized	70,836,597	1,154	0.0163	37.5 (33.1–41.5)	44.7 (40.8–48.3)	45.3 (41.2–49.2)
Fully immunized	92,622,083	360	0.0039	88.8 (87.4–90.0)	90.3 (89.1–91.4)	90.2 (88.9–91.4)
Confirmed death						
Unvaccinated	621,426,477	2,786	0.0045	—	—	—
Partially immunized	70,854,187	847	0.0120	39.8 (34.4–44.7)	45.7 (40.9–50.2)	46.0 (40.7–50.8)
Fully immunized	92,514,261	409	0.0044	84.4 (82.4–86.2)	86.3 (84.5–87.8)	86.7 (84.9–88.3)

* Participants were classified into three groups: those who were unvaccinated, those who were partially immunized (≥14 days after receipt of the first vaccine dose and before receipt of the second dose), and those who were fully immunized (≥14 days after receipt of the second dose). The 13 days between vaccine administration and partial or full immunization were excluded from the at-risk person-time. ICU denotes intensive care unit.

† The analysis was adjusted for age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19.

‡ A stratified version of the extended Cox proportional-hazards model was fit to test the robustness of the estimates to model assumptions, with stratification according to age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19.

death. Among fully immunized persons, the adjusted vaccine effectiveness was 65.9% for Covid-19 and 87.5% for hospitalization, 90.3% for ICU admission, and 86.3% for death. The vaccine-effectiveness results were maintained in both age-subgroup analyses, notably among persons 60 years of age or older, independent of variation in testing and independent of various factors regarding vaccine introduction in Chile.

The vaccine-effectiveness results in our study are similar to estimates that have been reported in Brazil for the prevention of Covid-19 (50.7%; 95% CI, 35.6 to 62.2), including estimates of cases that resulted in medical treatment (83.7%; 95% CI, 58.0 to 93.7) and estimates of a composite end point of hospitalized, severe, or fatal cases (100%; 95% CI, 56.4 to 100).[27] The large confidence intervals for the trial in Brazil reflect the relatively small sample (9823 participants) and the few cases detected (35 cases that led to medical treatment and 10 that were severe). However, our estimates are lower than the vaccine effectiveness recently reported in Turkey (83.5%; 95% CI, 65.4 to 92.1),[27,28] possibly owing to the small sample in that phase 3 clinical trial (10,029 participants in the per-protocol analysis), differences in local transmission dynamics, and the predominance of older adults among the fully or partially immunized participants in our study. Overall, our results suggest that the CoronaVac vaccine had high effectiveness against severe disease, hospitalizations, and death, findings that underscore the

Table 3. Effectiveness of CoronaVac Vaccine in Preventing Covid-19 Outcomes among Cohort Participants 60 Years of Age or Older, According to Immunization Status.

Outcome and Immunization Status	Subgroup Cohort	Persons with Covid-19		Vaccine Effectiveness (95% CI)		
	No. of Person-Days	No. of Persons	Incidence Rate	Analysis Adjusted for Sex and Age	Analysis Adjusted for All Covariates*	Stratified Analysis†
			no. of events/ 1000 person-days		*percent*	
Covid-19						
Unvaccinated	75,707,905	15,597	0.2060	—	—	—
Partially immunized	35,675,604	8,333	0.2336	3.9 (0.9–6.8)	9.7 (6.9–12.4)	12.7 (9.8–15.5)
Fully immunized	66,563,272	7,510	0.1128	63.4 (62.0–64.6)	66.6 (65.4–67.8)	67.2 (66.0–68.4)
Hospitalization						
Unvaccinated	76,047,640	5,304	0.0697	—	—	—
Partially immunized	35,961,593	2,168	0.0603	29.2 (25.1–33.1)	35.0 (31.3–38.6)	38.6 (34.8–42.2)
Fully immunized	66,986,859	1,344	0.0201	83.4 (82.2–84.5)	85.3 (84.3–86.3)	85.4 (84.3–86.4)
Admission to ICU						
Unvaccinated	76,194,648	1,811	0.0238	—	—	—
Partially immunized	36,062,081	672	0.0186	38.2 (31.9–44.0)	44.5 (38.7–49.7)	47.0 (41.2–52.2)
Fully immunized	67,051,769	331	0.0049	87.5 (85.7–89.0)	89.2 (87.6–90.6)	89.3 (87.8–90.7)
Confirmed death						
Unvaccinated	76,169,386	1,999	0.0262	—	—	—
Partially immunized	36,053,806	768	0.0213	39.7 (33.8–45.1)	45.8 (40.4–50.7)	46.1 (40.5–51.2)
Fully immunized	67,045,620	402	0.0060	84.4 (82.3–86.2)	86.5 (84.6–88.1)	86.8 (85.0–88.4)

* The analysis was adjusted for age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19.

† A stratified version of the extended Cox proportional-hazards model was fit to test the robustness of the estimates to model assumptions, with stratification according to sex, age, coexisting conditions, nationality, and income.

potential of this vaccine to save lives and substantially reduce demands on the health care system.

Our study has at least three main strengths. First, we used a rich administrative health care data set, combining data from an integrated vaccination system for the total population and from the Ministry of Health FONASA, which covers approximately 80% of the Chilean population. These data include information on laboratory tests, hospitalization, mortality, onset of symptoms, and clinical history in order to identify risk factors for severe disease. Information on region of residence also allowed us to control for differences in incidence across the country. We adjusted for income and nationality, which correlate with socioeconomic status in Chile and are thus considered to be social determinants of health. The large population sample allowed us to estimate vaccine effectiveness both for one dose and for the complete two-dose vaccination schedule. It also allowed for a subgroup analysis involving adults 60 years of age or older, a subgroup that is at higher risk for severe disease[3] and that is underrepresented in clinical trials. Second, data were collected during a rapid vaccination campaign with high uptake and during a period with one of the highest community transmission rates of the pandemic, which allowed for a relatively short follow-up period and for estimation of the prevention of at least four essential outcomes: Covid-19 cases and related hospitalization, ICU admission, and death. Finally, Chile has the highest testing rates for Covid-19 in Latin America, universal health care access, and a standardized, public reporting system for vital statistics, which limited the number of undetected or unascertained cases and deaths.[14]

Our study has several limitations. First, as an observational study, it is subject to confounding. To account for known confounders, we adjusted the analyses for relevant variables that could affect vaccine effectiveness, such as age, sex, underlying medical conditions, region of residence, and nationality. The risk of misclassification bias that would be due to the time-dependent performance of the SARS-CoV-2 RT-PCR assay is relatively low, because the median time from symptom onset to testing in Chile is approximately 4 days (98.1% of the tests were RT-PCR assays). In this 4-day period, the sensitivity and specificity of the molecular diagnosis of Covid-19 are high.[38] However, there may be a risk of selection bias. Systematic differences between the vaccinated and unvaccinated groups, such as health-seeking behavior or risk aversion, may affect the probability of exposure to the vaccine and the risk of Covid-19 and related outcomes.[39,40] However, we cannot be sure about the direction of the effect. Persons may be hesitant to get the vaccine for various reasons, including fear of side effects, lack of trust in the government or pharmaceutical companies, or an opinion that they do not need it, and they may be more or less risk-averse. Vaccinated persons may compensate by increasing their risky behavior (Peltzman effect).[40] We addressed potential differences in health care access by restricting the analysis to persons who had undergone diagnostic testing, and we found results that were consistent with those of our main analysis.

Second, owing to the relatively short follow-up in this study, late outcomes may not have yet developed in persons who were infected near the end of the study, because the time from symptom onset to hospitalization or death can vary substantially.[3,15] Therefore, effectiveness estimates regarding severe disease and death, in particular, should be interpreted with caution. Third, during the study period, ICUs in Chile were operating at 93.5% of their capacity on average (65.7% of the patients had Covid-19).[32] If fewer persons were hospitalized than would be under regular ICU operation, our effectiveness estimates for protection against ICU admission might be biased downward, and our effectiveness estimates for protection against death might be biased upward (e.g., if patients received care at a level lower than would usually be received during regular health system operation).

Fourth, although the national genomic surveillance for SARS-CoV-2 in Chile has reported the circulation of at least two viral lineages considered to be variants of concern, P.1 and B.1.1.7 (or the gamma and alpha variants, respectively),[41] we lack representative data to estimate their effect on vaccine effectiveness (Table S2). Results from a test-negative design study of the effectiveness of the CoronaVac vaccine in health care workers in Manaus, Brazil, where the gamma variant is now predominant, showed that the efficacy of at least one dose of the vaccine against Covid-19 was 49.6% (95% CI, 11.3 to 71.4).[30] Although the vaccine-effectiveness estimates in Brazil are not directly comparable with our estimates owing to differences in the target population, the vaccination schedule (a window of 14 to 28 days between doses is recommended in Brazil[42]), and immunization status, they highlight the importance of continued vaccine-effectiveness monitoring.

Overall, our study results suggest that the CoronaVac vaccine was highly effective in protecting against severe disease and death, findings that are consistent with the results of phase 2 trials[23,24] and with preliminary efficacy data.[27,28]

The research protocol was approved by the Comité Ético Científico Clínica Alemana Universidad del Desarrollo. The study was considered exempt from informed consent; no human health risks were identified. Research analysts are employees of the Chilean Ministry of Health; our use of data follows Chilean law 19.628 on private data protection.

Presented in part at the World Health Organization (WHO) Strategic Advisory Group of Experts on Immunization (SAGE) meeting on coronavirus disease 2019 (Covid-19) vaccines, April 29, 2021, and the WHO Vaccines Prioritization Working Group, April 21, 2021.

Supported by grants from the Agencia Nacional de Investigación y Desarrollo (ANID) Millennium Science Initiative Program, Millennium Nucleus Center for the Discovery of Structures in Complex Data (MIDAS) (NCN17_059, to Dr. Jara), and Millennium Initiative for Collaborative Research in Bacterial Resistance (MICROB-R) (NCN17_081, to Drs. Undurraga and Araos); the Advanced Center for Chronic Diseases, ANID Fondo de Financiamiento de Centros de Investigación en Áreas Prioritarias (FONDAP) (15130011, to Dr. Araos); and the Research Center for Integrated Disaster Risk Management ANID FONDAP (15110017, to Dr. Undurraga).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

Owing to data privacy regulations, the individual-level data in this study cannot be shared (Law N19.628). Aggregate data on vaccination and incidence are publicly available at https://github.com/MinCiencia/Datos-COVID19/.

We thank the attendees of the WHO meeting and working group for suggestions and comments; Drs. Ira Longini (University of Florida), Daniel Cohen (Tel Aviv University), Richard Peto (University of Oxford), Elizabeth Miller (Public Health England), and Myron M. Levine (University of Maryland); Drs. Catterina Ferreccio (Pontificia Universidad Católica de Chile), Ximena Aguilera (Universidad del Desarrollo), Maria T. Valenzuela (Universidad de Los Andes), Pablo Vial (Universidad del Desarrollo), Gonzalo Valdivia (Pontificia Universidad Católica de Chile), Fernando Otaiza (Ministerio de Salud), and Alvaro Erazo (Pontificia Universidad Católica de Chile), from the Chilean Covid-19 Advisory Group, for advice on the study design and interpretation of results; and Drs. José R. Zubizarreta (Harvard University) and Erika M.C. D'Agata (Brown University) for thoughtful suggestions.

REFERENCES

1. Dong E, Du H, Gardner L. An interactive Web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 2020;20:533-4.
2. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. JAMA 2020;324:782-93.
3. Verity R, Okell LC, Dorigatti I, et al. Estimates of the severity of coronavirus disease 2019: a model-based analysis. Lancet Infect Dis 2020;20:669-77.
4. Guan W-J, Ni Z-Y, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708-20.
5. Li Y, Campbell H, Kulkarni D, et al. The temporal association of introducing and lifting non-pharmaceutical interventions with the time-varying reproduction number (R) of SARS-CoV-2: a modelling study across 131 countries. Lancet Infect Dis 2021;21:193-202.
6. Walker PGT, Whittaker C, Watson OJ, et al. The impact of COVID-19 and strategies for mitigation and suppression in low- and middle-income countries. Science 2020;369:413-22.
7. Ledford H, Cyranoski D, Van Noorden R. The UK has approved a COVID vaccine — here's what scientists now want to know. Nature 2020;588:205-6.
8. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med 2020;383:2603-15.
9. Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet 2021;397:99-111.
10. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med 2021;384:403-16.
11. Logunov DY, Dolzhikova IV, Shcheblyakov DV, et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. Lancet 2021;397:671-81.
12. Tanne JH. Covid-19: FDA panel votes to approve Pfizer BioNTech vaccine. BMJ 2020;371:m4799.
13. Zimmer C, Corum J, Wee S-L. Coronavirus vaccine tracker. New York Times, June 10, 2021 (https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html).
14. Our World in Data. Coronavirus pandemic (COVID-19). 2021 (https://ourworldindata.org/coronavirus).
15. Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA Covid-19 vaccine in a nationwide mass vaccination setting. N Engl J Med 2021;384:1412-23.
16. Vasileiou E, Simpson CR, Robertson C, et al. Effectiveness of first dose of COVID-19 vaccines against hospital admissions in Scotland: national prospective cohort study of 5.4 million people. February 19, 2021 (https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3789264). preprint.
17. Thompson MG, Burgess JL, Naleway AL, et al. Interim estimates of vaccine effectiveness of BNT162b2 and mRNA-1273 COVID-19 vaccines in preventing SARS-CoV-2 infection among health care personnel, first responders, and other essential and frontline workers — eight U.S. locations, December 2020–March 2021. MMWR Morb Mortal Wkly Rep 2021;70:495-500.
18. Daniel W, Nivet M, Warner J, Podolsky DK. Early evidence of the effect of SARS-CoV-2 vaccine at one medical center. N Engl J Med 2021;384:1962-3.
19. Britton A, Jacobs Slifka KM, Edens C, et al. Effectiveness of the Pfizer-BioNTech COVID-19 vaccine among residents of two skilled nursing facilities experiencing COVID-19 outbreaks — Connecticut, December 2020–February 2021. MMWR Morb Mortal Wkly Rep 2021;70:396-401.
20. Doshi P. Will covid-19 vaccines save lives? Current trials aren't designed to tell us. BMJ 2020;371:m4037.
21. Lopalco PL, DeStefano F. The complementary roles of phase 3 trials and post-licensure surveillance in the evaluation of new vaccines. Vaccine 2015;33:1541-8.
22. Gao Q, Bao L, Mao H, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science 2020;369:77-81.
23. Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021;21:181-92.
24. Wu Z, Hu Y, Xu M, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021;21:803-12.
25. Cohen J, Moutinho S. Third time's the charm? Brazil scales back efficacy claims for COVID-19 vaccine from China. Science. January 12, 2021 (https://www.sciencemag.org/news/2021/01/third-time-s-charm-brazil-scales-back-efficacy-claims-covid-19-vaccine-china).
26. Baraniuk C. What do we know about China's covid-19 vaccines? BMJ 2021;373:n912.
27. Sinovac. Summary of clinical trial data of Sinovac's COVID-19 vaccine (CoronaVac). 2021. (In Chinese) (http://www.sinovacbio.com/?optionid=754&auto_id=927).
28. Tanriover MD, Doğanay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. Lancet 2021 July 8 (Epub ahead of print).
29. Centers for Disease Control and Prevention. SARS-CoV-2 variant classifications and definitions. 2021 (https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fcases-updates%2Fvariant-surveillance%2Fvariant-info.html).
30. Hitchings MDT, Ranzani OT, Scaramuzzini Torres MS, et al. Effectiveness of CoronaVac in the setting of high SARS-CoV-2 P.1 variant transmission in Brazil: a test-negative case-control study. May 1, 2021 (https://www.medrxiv.org/content/10.1101/2021.04.07.21255081v2). preprint.
31. Shepherd A. Covid-19: Chile joins top five countries in world vaccination league. BMJ 2021;372:n718.
32. Cifras Oficiales COVID-19. Ministerio de Salud, plan de acción coronavirus COVID-19, 2020 (https://www.gob.cl/coronavirus/cifrasoficiales/).
33. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. JAMA 2020;323:2052-9.
34. Bhargava A, Fukushima EA, Levine M, et al. Predictors for severe COVID-19 infection. Clin Infect Dis 2020;71:1962-8.
35. Numbers K, Brodaty H. The effects of the COVID-19 pandemic on people with dementia. Nat Rev Neurol 2021;17:69-70.
36. Therneau TM. A package for survival analysis in R: R package version 3.1-12. Rochester, MN: Mayo Clinic, April 2021 (https://cran.r-project.org/package=survival/survival.pdf).
37. R Development Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2019.
38. Jara A, Undurraga EA, Araos R. Tool for estimating the probability of having COVID-19 with one or more negative RT-PCR results. January 24, 2021 (https://www.medrxiv.org/content/10.1101/2021.01.16.21249939v1). preprint.
39. Lipsitch M, Jha A, Simonsen L. Observational studies and the difficult quest for causality: lessons from vaccine effectiveness and impact studies. Int J Epidemiol 2016;45:2060-74.
40. Trogen B, Caplan A. Risk compensation and COVID-19 vaccines. Ann Intern Med 2021;174:858-9.
41. Ministerio de Salud. Reporte circulación de variantes SARS-CoV-2 en Chile. Santiago, Chile: Departamento de Epidemiología Ministerio de Salud, 2021.
42. Ministério da Saúde. Informe técnico: campanha nacional de vacinação contra a Covid-19. January 19, 2021 (https://www.conasems.org.br/wp-content/uploads/2021/01/1611078163793_Informe_Tecnico_da_Campanha_Nacional_de_Vacinacao_contra_a_Covid_19-1.pdf).

5.9. Estudo com 60 milhões de brasileiros mostra efetividade da CoronaVac acima de 70% contra hospitalizações e mortes, inclusive entre idosos

Uma pesquisa realizada com 60,5 milhões de brasileiros vacinados entre janeiro e junho de 2021 mostrou que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, tem uma efetividade superior a 70% para evitar casos graves, internações em Unidades de Terapia Intensiva (UTIs) e mortes causadas por Covid-19, inclusive entre idosos. O estudo, que analisou a CoronaVac e a vacina da Astrazeneca/Fiocruz, é o maior já realizado no Brasil sobre a efetividade da vacinação contra o SARS-CoV-2.

Do total de pessoas avaliadas que haviam completado o esquema vacinal com CoronaVac (ou seja, tomado as duas doses), 72,6% apresentaram menor risco de hospitalização, 74,2% menor risco de admissão em UTI e 74% menor risco de morte. Em relação às pessoas entre 60 e 89 anos, a efetividade da vacina foi ainda melhor: 84,2% contra hospitalizações, 80,8% contra internações em UTI e 76,5% contra mortes.

O estudo foi realizado por pesquisadores das universidades federais da Bahia e de Ouro Preto, da Universidade de Brasília, da Universidade Estadual do Rio de Janeiro, da London School of Hygiene & Tropical Medicine e da Fundação Oswaldo Cruz Fiocruz. As conclusões foram publicadas no artigo "The effectiveness of Vaxzevria and CoronaVac vaccines: A nationwide longitudinal retrospective study of 61 million Brazilians (VigiVac-COVID19)", na plataforma de preprints MedRxiv.

Dos 60,5 milhões de brasileiros analisados no estudo, 21,9 milhões (36,2%) foram imunizados com a CoronaVac, e 38,6 milhões (63,8%) com a vacina da AstraZeneca/Fiocruz. Ao todo, 26,8 milhões de pessoas (44,4% do total) tinham 60 anos ou mais.

Para determinar a efetividade das vacinas em evitar casos graves de Covid-19, os pesquisadores confrontaram as informações de populações vacinadas com os dados nacionais do Sistema de Vigilância Epidemiológica da Gripe (SIVEP-Gripe), que reúne os

casos notificados de hospitalizações e mortes causadas por vírus respiratórios, como é o caso do SARS-CoV-2.

O levantamento é extremamente importante não apenas pelo número elevado de indivíduos analisados, como por se tratar do primeiro levantamento feito nacionalmente para aferir a efetividade vacinal – que não é a mesma coisa que eficácia. Enquanto a investigação da eficácia é feita em condições ideais e controladas, por vezes em laboratório, a análise da efetividade vacinal é baseada em dados do mundo real, onde a vacina é colocada à prova diante de um conjunto diverso de pessoas, em diferentes condições.

Outro estudo de efetividade feito em relação à CoronaVac foi o Projeto S, realizado pelo Butantan em Serrana, interior de São Paulo. Por meio dele, a população do município (quase 28 mil adultos) foi vacinada entre fevereiro e abril de 2021. A pesquisa concluiu que o imunizante causou uma redução de 80% no número de casos sintomáticos de Covid-19, de 86% nas internações e de 95% nos óbitos. Além disso, mostrou que com uma cobertura vacinal de aproximadamente 75% da população adulta a pandemia pode ser controlada.

A eficácia da CoronaVac foi comprovada no Brasil por meio de um estudo clínico de fase 3 com 13 mil voluntários, todos profissionais da saúde, população altamente exposta à Covid-19. Os resultados finais demonstraram que a eficácia geral do imunizante pode chegar a 62,3% quando o intervalo entre a primeira e a segunda dose é de 21 a 28 dias. Os dados foram divulgados na plataforma de preprints SSRN, ligada à revista The Lancet, e estão em processo de revisão por pares.

Publicado em: 25/08/2021

The effectiveness of Vaxzevria and CoronaVac vaccines: A nationwide longitudinal retrospective study of 61 million Brazilians (VigiVac-COVID19).

Short Title: **Effectiveness of Vaxzevria and CoronaVac vaccines in Brazil**

Thiago Cerqueira-Silva [1,2], Vinicius de Araújo Oliveira, M.D. [,1,2,3], Julia Pescarini, Ph.D. [3,4], Juracy Bertoldo Júnior [2,3], Tales Mota Machado [5], Renzo Flores Ortiz, Ph.D. [3], Gerson Penna, M.D., Ph.D. [6], Maria Yury Ichihara, M.D., Ph.D. [3], Jacson Venâncio de Barros [7], Viviane S. Boaventura, M.D., Ph.D. [1,2], Mauricio L. Barreto, M.D., Ph.D. [2,3], Guilherme Loureiro Werneck, M.D., Ph.D. [8], Manoel Barral-Netto, M.D., Ph.D. [1,2,3]

Affiliations:

1.LIB and LEITV Laboratories, Instituto Gonçalo Moniz, Fiocruz, Salvador, Bahia, Brazil;

2. Universidade Federal da Bahia (UFBA), Salvador, Bahia, Brazil;

3. Center for Data Integration and Knowledge for Health (Cidacs / IGM / Fiocruz), Salvador, Bahia, Brazil;

4. London School of Hygiene and Tropical Medicine, London, United Kingdom

5. Universidade Federal de Ouro Preto, Ouro Preto, Brazil;

6. Núcleo de Medicina Tropical, Universidade de Brasília. Escola Fiocruz de Governo, Fiocruz Brasilia. Brasilia, DF, Brazil;

7. Department of Health Informatics, Ministry of Health, Brasília, Brazil

8. Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

Corresponding author:

Manoel Barral-Netto, MD

manoel.barral@fiocruz.br

Abstract

Background

High rates of virus transmission and the presence of variants of concern can affect vaccine effectiveness (VE). Both conditions occur in low-income countries, which primarily use viral vector or inactivated virus vaccine technologies. However, few VE analyses have been conducted in such countries, and most lack the power to evaluate effectiveness in subgroups, such as the elderly.

Methods

The present retrospective cohort study evaluated the effectiveness of Vaxzevria and CoronaVac vaccines for COVID-19-related infection in 60,577,870 Brazilian vaccinees from January 18 to June 30, 2021.

Study outcomes included documented infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Covid-19–related hospitalization, ICU admission and death. We estimated VE for each outcome as one minus the hazard ratio using Cox regression adjusted for age, sex, Brazilian deprivation index, and month/region of dose administration.

Results

Vaccination with Vaxzevria or CoronaVac was found to be effective against SARS-CoV-2 infection and highly effective against hospitalization, ICU admission and death in individuals up to 79 years. From 80-89 years of age, 91.2 (95CI: 89.1-92.9) VE against death was seen in Vaxzevria-vaccinated individuals versus 67.3 (95CI: 63.6-70.6) for Coronvac. Above 90 years, 70.5 (95CI: 51.4-82.1) protection was conferred to Vaxzevria-vaccinated individuals versus 35.4 (95CI: 23.8-45.1) in Coronavac-vaccinated individuals

Conclusions

Both vaccines demonstrated overall effectiveness against severe COVID-19 up to 80 years of age. Our results suggest that individuals aged 90 years or older may benefit from an expedited third booster dose. Ongoing evaluations, including any additional vaccines authorized, are crucial to monitoring long-term vaccine effectiveness.

Background

Several COVID-19 vaccines have proved efficacious, and many of them are being extensively used around the world.[1–4] While high-income countries preferentially administer mRNA-based vaccines, lower- and middle-income countries have employed vaccines based on viral vectors or inactivated virus technologies. A timely evaluation of the effectiveness of the currently available vaccines across different regions is essential for a comprehensive understanding of vaccine impact, considering significant variations in vaccination schedules, virus transmission and the emergence of viral variants, in addition to social and cultural standards and local health system conditions.

Brazil is one of the countries most affected by the pandemic, with high rates of transmission. The Brazilian COVID-19 vaccination program initially relied on the vaccines Vaxzevria/Fiocruz (previously Oxford-AstraZeneca or ChAdOx-1), approved in 181 countries, and Sinovac's CoronaVac/Butantan, approved in 39 countries.[5] The recommended interdose interval in Brazil for Vaxzevria is 12 weeks versus 2-4 weeks for CoronaVac. The period between doses of Vaxzevria has varied in several countries.[7] However, CoronaVac has been applied at distinct intervals,[1,8] making direct comparisons difficult. Additionally, several early publications on vaccine effectiveness (VE) evaluated only the initial dose or were limited to analyzing effectiveness against symptomatic infection[9,10] and hospitalization[10,11], i.e., ICU admission and death were not addressed.

Nationwide evaluations of the effectiveness of COVID-19 vaccines in Brazil offers advantages, as this country's large population is distributed throughout several regions with considerable differences in socio-economic aspects and access to medical facilities. Nonetheless, data collection systems are identical throughout the entire country, offering a comprehensive source of data to perform a countrywide VE evaluations. The COVID-19 vaccination campaign was initiated nationwide on January 18, 2021. By June, a large number

of vaccinees had received either Vaxzevria/Fiocruz or CoronaVac/Butantan vaccines, allowing for a detailed evaluation of the effectiveness of both vaccines while considering several outcomes and stratified age ranges, making it possible to examine in detail specific age effects previously not investigated.

A significant issue regarding the VE of vaccines against COVID-19 is the degree of circulation of distinct SARS-CoV-2 variants of concern (VOC) in different regions. During the course of the present study, the Gamma variant was the most frequent in all regions of Brazil.[12] Importantly, the literature contains few reports on the VE of Vaxzevria and Coronavac against the Gamma variant.[1,10,13]

The present study aimed to evaluate the effectiveness of Vaxzevria and Coronavac vaccines in 60,577,870 Brazilian vaccinee with respect to several different outcomes: COVID-19 related infection, hospitalization, ICU admission and death, between January 18 and June 30, 2021.

Methods

Study design and datasets

We conducted a retrospective cohort using individual-level information on demographic, clinical characteristics, and SARS-COV-2 laboratory tests from the Brazilian federal health registries. The Brazilian Ministry of Health Department of Informatics (DATASUS) provided unidentified datasets of the COVID-19 Vaccination Campaign dataset (SI-PNI), the Acute Respiratory Infection Suspected Cases dataset (e-SUS Notifica), and the National Epidemiological Surveillance System registry for Severe Acute Respiratory Infection/Illness (SIVEP-Gripe). A key-coded individual identification number present in the three datasets was used for a deterministic linkage and then removed from the resulting linked dataset used

in our analyses. No personally identifiable data was accessed at any stage. Codebooks, scripts and public dataset version will be available at https://vigivac.fiocruz.br

SI-PNI is a data warehouse run by DATASUS with all the vaccine doses administered by health services in Brazil. From SI-PNI, we extracted information on the COVID-19 vaccine received either Sinovac CoronaVac or Vaxzevria (under the names AstraZeneca/Fiocruz or Covishield/ChAdOx1-S), and the dates of the first and second doses. Overall and age-specific Brazilian population estimates for 2021 corrected the all-cause deaths reported in 2020 overall and age were retrieved from the Brazilian Institute of Geography and Statistics.[14] Open version of the SI-PNI dataset is available at opendatasus-SI PNI.

The e-SUS Notifica is a national online health surveillance information system where acute respiratory infections cases and COVID-19 suspected or confirmed cases are registered. and has been used as a data source for epidemiological research.[15] Open version of e-SUS Notifica is available at opendatasus-eSUS Notifica.

SIVEP-Gripe is the national system used to register SARI-related hospitalizations and deaths caused by influenza or other respiratory viruses. The system is a registry for new respiratory infections since the H1N1 pandemic in 2009 and widely used as a source for epidemiological studies.[16–18] All COVID-19 related SARI hospitalizations and deaths (independent of hospitalization) are registered in the system. Open version of the 2021 SIVEP-Gripe dataset is available at opendatasus-SIVEP

From both SIVEP-Gripe and eSUS-Notifica, we extracted information on the date of symptom onset, RT-PCR, and antigen test results for SARS-CoV-2, and from SIVEP-Gripe, we got data of hospitalization, admission to ICU, and hospitalization outcome (discharge or death).

Study population

We included all individuals who received the COVID-19 vaccine first dose between January 18th, 2021, and June 30th, 2021. The study individuals were followed retrospectively to assess infection, hospitalization, admission to ICU, and death with a laboratory-confirmed diagnosis of SARS-CoV-2 up to June 30th, 2021.

We excluded individuals (i) vaccinated with vaccines besides Vaxzevria or CoronaVac, (ii) with inconsistent vaccine records (i.e., individuals who received the second dose without the first dose, received doses from different vaccines or interval between doses less than 14 days), (iii) with confirmed COVID-19 before the date of vaccine administration, and (iv) with missing data for essential covariates (i.e., sex or age).

Exposure and outcomes

We defined vaccination status for each vaccine based on the time elapsed since the administration of a vaccine dose:

1. ≤13 days after the first dose (the reference period)
2. ≥14 days after the first dose and without the second dose (partially vaccinated)
3. ≥14 days after the second dose (fully vaccinated)

We defined the period up to 13 days after the first dose as the reference period for VE estimation based on results of a Phase III randomized controlled trial[8] and three test-negative studies.[11,19,20] The time-lapsed between the date of the first dose and the development of an effective immune response is used to detect bias in test-negative case-control studies to estimate vaccine effectiveness, the theoretical frame for such use has been discussed by Hitchings et al.[21] We also analyzed vaccine effectiveness for 1 to 13 days after the second dose, with the results presented in supplementary table S1).

Laboratory confirmation of COVID-19 with a positive RT-PCR or antigen test result) was required for inclusion in the analyses. The outcomes analyzed were infection, hospitalization, admission to an intensive care unit (ICU), and death by COVID-19. We considered the time

between day one of the first or second vaccination up to the symptom's onset for each outcome. Individuals whose symptoms started on the same day of the first vaccination dose were assigned one day of follow-up time. Death was considered at any time regardless of prior hospitalization. ICU admission was considered at any time point between the admission and the discharge or death dates.

Statistical analyses

In the primary analysis, we used a Cox regression model to estimate the hazard ratio (HR) of COVID-19 infection, hospitalization, ICU admission, and death for partially and fully vaccinated individuals. The model was adjusted for age, sex, region of residence, socioeconomic status, and month of the 1st dose. We used the Brazilian Deprivation Index (*Índice Brasileiro de Privação*-IBP), a municipality-level measure of material deprivation, as an indicator of socioeconomic status.[21] We estimated vaccine effectiveness (VE) as 1-HR, obtained from a model including all covariates, and reported as a percentage. We also reported the crude VE for each outcome. In addition, we performed a stratified analysis by age groups (<60, 60–69, 70–79, 80–89, ≥90 years) to investigate whether VE was modified by age.

To assess the robustness of our findings, we repeated the principal analysis defining as the reference period the time elapsed up to 10 days after the date of the first dose, as it is expected that the vaccines' protection increases with time. Additionally, we examined the VE for hospitalization, ICU admission and death using clinical suspected cases besides laboratory confirmed ones.

Analyses were performed using the R statistical software (R Core Team) and its H2O package.[23,24] Descriptive statistics were presented as frequencies and percentages. We used

the 95% confidence intervals (CI) of the estimated measures of association for interpreting the findings.

RESULTS

From January 18 to June 30, 2021, 61,783,842 individuals received at least one dose of one of the two COVID-19 vaccines analyzed in this study, and 60,577,870 (98.1%) met the eligibility criteria and were included in the analysis (Figure 1). The majority (63.8%, n=38,664,633 individuals) received at least one dose of Vaxzevria and the remaining (36.2%, n=21,933,237 individuals) received at least one dose of CoronaVac. The majority of our cohort comprised women (56.1%) and individuals aged 60 years or older (44.4%). Compared to individuals that received CoronaVac, individuals that received Vaxzevria were younger (29.3% vs. 70.9% of individuals aged 60 years or older), and a lower proportion had completed the full vaccine schedule (10.6% vs. 82.7%). Vaccination with CoronaVac occurred mainly from January to April 2021, while Vaxzevria was administered predominantly after March 2021 (Figure 2). Among those who received the second dose, the median time between the first and second doses was 85 days (IQR 83–90) for Vaxzevria and 27 days (IQR 21–28) for CoronaVac. Individuals who received at least one dose of Vaxzevria or CoronaVac were mostly women (54.6% vs. 58.7% respectively) and from the southeast region of the country (44.1% vs. 46.3%, respectively) (Table 1).

Table 2 shows the COVID-19 VE analysis results, including number of events and incidence rate per 1000 person-days and supplementary table S1 shows the crude and adjusted VE analysis . We observed that individuals with full vaccination schedule (i.e., ≥ 14 days after the second dose) with Vaxzevria had a 70.0% (95% CI 68.6 to 71.3) lower risk of infection, 86.8% (95% CI 85.2 to 88.2) lower risk of hospitalization, 88.1% (95% CI 85.4 to 90.3) lower risk of ICU admission, and 90.2% (95% CI 88.3 to 91.8) lower risk of death. Partial vaccination (i.e., ≥14 days after the first dose up to the second dose) with Vaxzeria was

associated with a 32.7% lower risk of infection (95% CI 31.9 to 33.5) and at least 50% lower risk of hospitalization (51.7%; 95% CI 50.4 to 52.9), ICU admission (53.6%; 95% CI 51.4 to 55.6), and death (49.3%; 95% CI 47.0 to 51.5). Complete vaccination with CoronaVac was associated with a 54.2 (95% CI 53.4-55.0) lower risk of infection, 72.6% (95% CI 71.6 to 73.6) lower risk of hospitalization, 74.2% (95% CI 72.6 to 75.7) lower risk of ICU admission, and 74.0% (95% CI 72.6 to 75.3) lower risk of death. Partial vaccination with CoronaVac was associated with less than 50% of reduction in the risk of infection (16.2%; 95% CI 15.1 to 17.4), hospitalization (26.5%; 95% CI 24.6 to 28.4), ICU admission (28.1%; 95% CI 24.9 to 31.1), and death (29.4%; 95% CI 26.7 to 32.0).

When stratifying the analysis by age, complete vaccination with Vaxzevria or CoronaVac presented a similar VE within all age groups, with the exception among individuals aged 90 years or older (Table S2, Figure 3).

In the analysis using the reference period of up to 10 days after the first dose, we found VE point and interval estimates similar to those found in the primary analysis for both Vaxzeria and Coronavac vaccines (Table S3). The results using all clinical suspected and laboratory confirmed cases for the outcomes of hospitalization, ICU admission and death were qualitatively equal to those found in primary analysis (Table S4).

DISCUSSION

Here we present nationwide results on the effectiveness of vaccination with CoronaVac/Butantan and Vaxzevria/Fiocruz after the first six months of the vaccination campaign in Brazil. Analyzing data from almost 61 million individuals vaccinated with at least one dose, our results demonstrate strong evidence of 70.0% and 54.2% protection against infection after full vaccination with Vaxzevria and CoronaVac, respectively. Vaxzevria offered approximately 90% effectiveness against hospitalization, ICU admission

and death, while CoronaVac provided approximately 75% protection following full vaccination.

Our findings regarding the Coronovac/Butantan vaccine are compatible with a previous Brazilian efficacy study[24], but lower than the 83.5% protection reported by a Turkish efficacy trial.[8] The effectiveness determined by a cohort study in Chile was also higher than our findings for infection (66.5% vs. 54.2%) as well as hospitalization (87.5% vs. 72.6%). Differences between the study in Chile and the present analyses of Brazilian vaccinees may be partially explained by the higher frequency of younger individuals in the Chile study (51.2% vs. 29.1% of individuals younger than 60 years old). During the vaccination campaign, Brazil experienced health system collapse in several states, which may have influenced death rates, especially between February and May, likely affecting CoronaVac estimates more markedly due to its greater availability of this vaccine in the early stages of the vaccination program. Another reason for these differences could be the increased circulation of the Gamma lineage detected in these countries, which has been estimated at 28.6% in Chile and 69.6% in Brazil during both study periods.[1,12] In plasma samples obtained from individuals fully vaccinated with CoronaVac, a reduced capacity to neutralize the Gamma variant was observed.[1] Furthermore, 9.9% of the Brazilian population was fully vaccinated from January to May 2021, compared to almost 35.4% of Chile's population. This may have contributed to lower viral transmission in Chile compared to Brazil.[1]

For Vaxzevria, our findings of 70.0% effectiveness against infection exceeded the levels of 66.7% effectiveness reported in a combined analysis of four clinical trials conducted in the UK, South Africa, and Brazil.[7] Effectiveness against hospitalization was consistent with the 80% and 89% protection observed in studies in Scotland[3] and England,[11] respectively. Additionally, our findings support the high level of protection offered by Vaxzevria despite the abundant circulation of the Gamma variant in Brazil during the period studied. Few

studies have reported on the VE of Vaxzevria in populations infected by VOCs.[1,9,10,13,20] Studies analyzing effectiveness against VOCs have mainly focused on protection against symptomatic infection or hospitalization.[9,10,13] Taken together, the findings reported herein combined with data in the literature confirm a consistently high rate of protection against moderate to severe COVID-19 in real-world studies, despite abundant circulation of VOCs.

Protection was shown to vary according to age group. The VE of CoronaVac/Butantan was close to 80% against death in individuals aged up to 79 years of age. However, a reduction in effectiveness was observed after 80 years of age, with only 35.4% protection against death seen in individuals over 90. In contrast, the Vaxzevria/Fiocruz vaccine achieved close to 90% protection against death in individuals aged less than 90 years, while a VE of 70.5% was found in those older than 90 years of age. It is reasonable to attribute the observed reduction in effectiveness to immunosenescence, which is commonly associated with a higher frequency of comorbidities, and may imply higher death rates. In the context of limited vaccine availability, the precise identification of age limits at which point immune protection becomes impaired can provide valuable evidence to inform public health measures. Considering the current scenario in Brazil, our findings demonstrate the eventual need for a vaccine booster dose in individuals aged 80 years or older who received CoronaVac, as well as for individuals over 90 years immunized with Vaxzevria.

The differences evidenced in effectiveness between Vaxzevria and CoronaVac may be related to the distinct technologies used be each of these two products, as well as how they influence immunogenicity. Both vaccines analyzed herein activate immunological mechanisms and trigger a neutralizing antibody response against viral particles. However, CoronaVac, a whole-cell inactivated vaccine, elicits a less potent cellular response than Vaxzevria, an adenoviral-vectored vaccine.[25] Additionally, Vaxzevria was shown to induce a higher peak neutralizing antibody response than CoronaVac.[27] Thus, the intrinsic

characteristics of each formulation may serve to explain differences observed in both clinical trials and vaccine effectivity studies.[1,26,28]

A relevant strength of our study is its large sample size, due to the use of the complete dataset covering the Brazilian COVID-19 vaccination campaign from January to June 2021. This large sample allowed us to identify the age limits in which immune protection becomes impaired, especially with regard to CoronaVac. Sensitivity analyses further confirmed the robustness of our findings. However, our study is also subject to some limitations. First, as VE was estimated using observational data, our analysis is subject to data availability and, therefore, to potential confounders. Although our analyses were not controled for comorbidities, crude and adjusted VE estimates were similar. In addition, comorbidities have been identified as the causal pathway between age and COVID-19 severity. Therefore, by controlling for age, we are also indirectly controlling for comorbidities.[29] Second, in contrast to many VE studies, the reference period used herein for comparison purposes was 1-13 days after vaccination. Although using early post-vaccination as a reference may underestimate VE, previous studies have used a similar approach and obtained VE results similar to those found in clinical trials.[30,31] The early post-vaccination period can also be used as a bias indicator related to differences in SARS-CoV-2 infection risk. Additionally, the effectiveness results of the present report are similar, in the pertinent age ranges, to reports on both vaccines using distinct approaches.[1,19,20] Finally, we also performed sensitivity analysis, which demonstrated similar results when a 0-10 day reference period was applied.

Using the data available in Brazil, we estimated overall VE for each vaccine evaluated as well as by age group. Vaxzevria/Fiocruz and CoronaVac/Butantan were both shown to be highly protective against severe COVID-19 in the population aged up to 80 years, yet due to decreased VE an early booster dose may be considered for those over 80 years of age who received CoronaVac, and especially for individuals aged over 90 years regardless of which of

these two vaccines were administered. Despite high population adherence, the vaccination campaign is evolving unevenly throughout Brazil, and continuous monitoring of VE in the current context may provide sound evidence to inform public health measures.

ETHICAL CONSIDERATIONS

The Brazilian National Commission in Research Ethics approved the research protocol (CONEP approval number 4.921.308). The study was considered exempt from informed consent; no human health risks were identified. All work presented here used unidentified secondary data in accordance with the Brazilian Personal Data Protection General Law (LGPD). Data was manipulated in a secure computing environment, ensuring protection against data leakage and records reidentification.

DECLARATION OF INTERESTS

VO, VB, MB, and MB-N are employees from Fiocruz, a federal public institution, which manufactures Vaxzevria in Brazil, through a full technology transfer agreement with AstraZeneca. Fiocruz allocates all its manufactured products to the Ministry of Health for the public health service (SUS) use. All other authors report no potential competing interest.

FUNDING

This study was partially supported by a donation from the "Fazer o bem faz bem" program.

DATA SHARING

We used third-party data, provided by the Brazilian Ministry of Health. Any request for access to the data shall be directed to DATASUS - Ministry of Health Brazil:

https://datasus.saude.gov.br/

ACKNOWLEDGMENTS

The authors thank DATASUS for its excellent work in providing unidentified databases. GW, MB, and MB-N are research fellows from CNPq, the Brazilian National Research Council. The authors would also like to thank Andris K. Walter for English language revision and manuscript copyediting assistance.

REFERENCES

1. Jara A, Undurraga EA, González C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. New England Journal of Medicine 2021;0(0):null.
2. Rossman H, Shilo S, Meir T, Gorfine M, Shalit U, Segal E. COVID-19 dynamics after a national immunization program in Israel. Nat Med 2021;27(6):1055–61.
3. Vasileiou E, Simpson CR, Shi T, et al. Interim findings from first-dose mass COVID-19 vaccination roll-out and COVID-19 hospital admissions in Scotland: a national prospective cohort study. The Lancet 2021;397(10285):1646–57.
4. Moghadas SM, Vilches TN, Zhang K, et al. The Impact of Vaccination on Coronavirus Disease 2019 (COVID-19) Outbreaks in the United States. Clinical Infectious Diseases [Internet] 2021 [cited 2021 Jul 31];(ciab079). Available from: https://doi.org/10.1093/cid/ciab079
5. Holder J. Tracking Coronavirus Vaccinations Around the World [Internet]. The New York Times. 2021 [cited 2021 Jul 31];Available from: https://www.nytimes.com/interactive/2021/world/covid-vaccinations-tracker.html
6. Ministério da Saúde, 2021. Plano nacional de operacionalização da vacinação contra a covid-19: 9ª edição [Internet]. Brasília, 2021. [cited 02/08/2021]. 113 p. Available from: https://www.gov.br/saude/pt-br/coronavirus/publicacoes-tecnicas/guias-e-planos/plano-nacional-de-vacinacao-covid-19
7. Voysey M, Clemens SAC, Madhi SA, et al. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. The Lancet 2021;397(10277):881–91.

8. Tanriover MD, Doğanay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. The Lancet 2021;398(10296):213–22.

9. Sheikh A, McMenamin J, Taylor B, Robertson C. SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness. The Lancet 2021;397(10293):2461–2.

10. Nasreen S, Chung H, He S, et al. Effectiveness of COVID-19 vaccines against variants of concern in Ontario, Canada. medRxiv 2021;2021.06.28.21259420.

11. Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. BMJ 2021;373:n1088.

12. Rede Genômica Fiocruz. FREQUENCIA DAS PRINCIPAIS LINHAGENS DO SARS-CoV-2 POR MÊS DE AMOSTRAGEM [Internet]. Genomahcov - Fiocruz. [cited 2021 Jul 31];Available from: http://www.genomahcov.fiocruz.br/frequencia-das-principais-linhagens-do-sars-cov-2-por-mes-de-amostragem/

13. Bernal JL, Andrews N, Gower C, et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. New England Journal of Medicine [Internet] 2021 [cited 2021 Jul 31];Available from: https://www.nejm.org/doi/10.1056/NEJMoa2108891

14. Victora PC, Castro PMC, Gurzenda S, Medeiros AC, França GVA, Barros PAJD. Estimating the early impact of vaccination against COVID-19 on deaths among elderly people in Brazil: Analyses of routinely-collected data on vaccine coverage and

mortality. EClinicalMedicine [Internet] 2021 [cited 2021 Aug 3];0(0). Available from: https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(21)00316-3/abstract

15. Lima FET, Albuquerque NLS de, Florencio S de SG, et al. Time interval between onset of symptoms and COVID-19 testing in Brazilian state capitals, August 2020. Epidemiol Serv Saude 2020;30(1):e2020788.

16. Ranzani OT, Bastos LSL, Gelli JGM, et al. Characterisation of the first 250 000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. The Lancet Respiratory Medicine 2021;9(4):407–18.

17. Oliveira EA, Colosimo EA, Silva ACS e, et al. Clinical characteristics and risk factors for death among hospitalised children and adolescents with COVID-19 in Brazil: an analysis of a nationwide database. The Lancet Child & Adolescent Health 2021;5(8):559–68.

18. Baqui P, Bica I, Marra V, Ercole A, Schaar M van der. Ethnic and regional variations in hospital mortality from COVID-19 in Brazil: a cross-sectional observational study. The Lancet Global Health 2020;8(8):e1018–26.

19. Ranzani OT, Hitchings MDT, Dorion M, et al. Effectiveness of the CoronaVac vaccine in the elderly population during a Gamma variant-associated epidemic of COVID-19 in Brazil: A test-negative case-control study. medRxiv 2021;2021.05.19.21257472.

20. Hitchings MDT, Ranzani OT, Dorion M, et al. Effectiveness of the ChAdOx1 vaccine in the elderly during SARS-CoV-2 Gamma variant transmission in Brazil. medRxiv 2021;2021.07.19.21260802.

21. Hitchings MDT, Lewnard JA, Dean NE, et al. Use of recently vaccinated individuals to detect bias in test-negative case-control studies of COVID-19 vaccine effectiveness. medRxiv 2021;2021.06.23.21259415.

22. Allik, Mirjam, Ramos, Dandara, Agranonik, Marilyn, et al. Small-area Deprivation Measure for Brazil: Data Documentation [Internet]. 2020 [cited 2021 Jul 31]; Available from: http://researchdata.gla.ac.uk/id/eprint/980.

23. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from: https://www.R-project.org/.

24. LeDell E, Gill N, Aiello S, Fu A, Candel A, Click C, et al. h2o: R Interface for the "H2O" Scalable Machine Learning Platform [Internet]. 2021. Available from: https://github.com/h2oai/h2o-3.

25. Palacios R, Batista AP, Albuquerque CSN, et al. Efficacy and Safety of a COVID-19 Inactivated Vaccine in Healthcare Professionals in Brazil: The PROFISCOV Study [Internet]. Rochester, NY: Social Science Research Network; 2021 [cited 2021 Jul 31]. Available from: https://papers.ssrn.com/abstract=3822780

26. Sadarangani M, Marchant A, Kollmann TR. Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. Nat Rev Immunol 2021;21(8):475–84.

27. Rogliani P, Chetta A, Cazzola M, Calzetta L. SARS-CoV-2 Neutralizing Antibodies: A Network Meta-Analysis across Vaccines. Vaccines 2021;9(3):227.

28. Folegatti PM, Ewer KJ, Aley PK, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. The Lancet 2020;396(10249):467–78.

29. Telle KE, Grøsland M, Helgeland J, Håberg SE. Factors associated with hospitalization, invasive mechanical ventilation treatment and death among all confirmed COVID-19 cases in Norway: Prospective cohort study. Scand J Public Health 2021;49(1):41–7.

30. Chodick G, Tene L, Rotem RS, et al. The Effectiveness of the Two-Dose BNT162b2 Vaccine: Analysis of Real-World Data. Clinical Infectious Diseases [Internet] 2021 [cited 2021 Jul 31];(ciab438). Available from: https://doi.org/10.1093/cid/ciab438

31. Chodick G, Tene L, Patalon T, et al. Assessment of Effectiveness of 1 Dose of BNT162b2 Vaccine for SARS-CoV-2 Infection 13 to 24 Days After Immunization. JAMA Netw Open 2021;4(6):e2115985.

TABLES AND FIGURES

Table 1. Demographic characteristics of individuals that received at the first dose of Vaxzevria and CoronaVac in Brazil between 18th January and 30th June 2021.

	Vaxzevria/Fiocruz			CoronaVac/Butantan		
	Persons with only one dose N=34,556,983 n (%)	Persons with two doses N=4,107,650 n (%)	Total N=38,664,633 n (%)	Persons with only one dose N=3,794,753 n (%)	Persons with two doses N=18,138,484 n (%)	Total N=21,933,237 n (%)
Sex (Female)	18,603,771 (53.8)	2,509,503 (61.1)	21,113,274 (54.6)	2,136,515 (56.3)	10,739,832 (59.2)	12,876,347(58.7)
Age group						
<20	279,896 (0.8)	18,880 (0.5)	298,776 (0.8)	36,246 (1.0)	57,185 (0.3)	93,431 (0.4)
20-29	2,369,858 (6.9)	284,973 (6.9)	2,654,831 (6.9)	294,281 (7.8)	832,301 (4.6)	1,126,582 (5.1)

30-39	3,935,033 (11.4)	427,267 (10.4)	4,362,300 (11.3)	351,089 (9.3)	1,204,701 (6.6)	1,555,790 (7.1)
40-49	7,143,476 (20.7)	386,696 (9.4)	7,530,172 (19.5)	988,384 (26.0)	1,091,683 (6.0)	2,080,067 (9.5)
50-59	12,198,475 (35.3)	280,890 (6.8)	12,479,365 (32.3)	671,336 (17.7)	863,722 (4.8)	1,535,058 (7.0)
60-69	7,899,957 (22.9)	751,488 (18.3)	8,651,445 (22.4)	631,203 (16.6)	5,211,550 (28.7)	5,842,753 (26.6)
70-79	401,161 (1.2)	591,043 (14.4)	992,204 (2.6)	611,335 (16.1)	6,701,411 (36.9)	7,312,746 (33.3)
80-89	284,210 (0.8)	1,234,312 (30.0)	1,518,522 (3.9)	163,675 (4.3)	1,712,040 (9.4)	1,875,715 (8.6)
≥90	44,917 (0.1)	132,101 (3.2)	177,018 (0.5)	47,204 (1.2)	463,891 (2.6)	511,095 (2.3)
Region of residence						
Central West	2,568,166 (7.4)	342,173 (8.3)	2,910,339 (7.5)	246,240 (6.5)	1,359,139 (7.5)	1,605,379 (7.3)

Northeast	825,655 (2.4)	1,074,931 (26.2)	1,900,586 (4.9)	769,299 (20.3)	4,412,161 (24.3)	5,181,460 (23.6)
North	2,453,059 (7.1)	507,337 (12.4)	2,960,396 (7.7)	242,527 (6.4)	1,165,657 (6.4)	1,408,184 (6.4)
Southeast	15,479,240 (44.8)	1,582,019 (38.5)	17,061,259 (44.1)	2,083,624 (54.9)	8,077,669 (44.5)	10,161,293 (46.3)
South	5,621,171 (16.3)	575,822 (14.0)	6,196,993 (16.0)	427,859 (11.3)	3,021,915 (16.7)	3,449,774 (15.7)
Missing	178,789 (0.5)	25,368 (0.6)	204,157 (0.5)	25,204 (0.7)	101,943 (0.6)	127,147 (0.6)
Brazilian Municipal Deprivation Index						
1	7,140,436 (20.7)	776,055 (18.9)	7,916,491 (20.5)	788,353 (20.8)	3,973,481 (21.9)	4,761,834 (21.7)

24

2	6,616,814 (19.1)	712,784 (17.4)	7,329,598 (19.0)	994,456 (26.2)	3,456,814 (19.1)	4,451,270 (20.3)
3	7,071,108 (20.5)	833,540 (20.3)	7,904,648 (20.4)	729,322 (19.2)	3,751,664 (20.7)	4,480,986 (20.4)
4	6,925,602 (20.0)	853,682 (20.8)	7,779,284 (20.1)	595,008 (15.7)	3,580,458 (19.7)	4,175,466 (19.0)
5	6,624,234 (19.2)	906,221 (22.1)	7,530,455 (19.5)	662,410 (17.5)	3,274,124 (18.1)	3,936,534 (17.9)
Missing	178,789 (0.5)	25,368 (0.6)	204,157 (0.5)	25,204 (0.7)	101,943 (0.6)	127,147 (0.6)

The study participants were included if they received first dose of CoronaVac of Vaxzevria between January 18 and June 30, 2021. The Brazilian Municipal Deprivation Index works as proxy for socioeconomic status.

Table 2. Vaccine effectiveness of Vaxzevria and CoronaVac in Brazil for COVID-19 infection, hospitalization, ICU admission, and death.

	Vaxzevria/Fiocruz				CoronaVac/Butantan			
	Person-days	Events	Incidence per 1000 person-days	VE % (95% CI)*	Person-days	Events	Incidence per 1000 person-days	VE % (95% CI)*
Infection								
Reference period	474,317,595	76,780	0,1619	Ref	272,340,929	47,523	0,1745	Ref
Partially vaccinated	1,183,986,976	119,195	0.1007	32.7 (31.9-33.5)	431,038,009	55,495	0.1287	16.2 (15.1-17.4)
Fully	98,266,804	6,271	0.0638	70.0 (68.6-	1,184,435,889	108,998	0.0920	54.2 (53.4-55.0)

vaccinated				71.3)				
Hospitalization								
Reference period	474,679,253	18,420	0.0389	Ref	272,540,206	15,080	0.0553	Ref
Partially vaccinated	1,189,453,888	20,998	0.0177	51.7 (50.4-52.9)	434047110	14,484	0.0334	26.5 (24.6-28.4)
Fully vaccinated	99,464,137	574	0.0058	86.8 (85.2-88.2)	1192845239	20,299	0.0170	72.6 (71.6-73.6)
ICU admission								
Reference period	474,760,394	6,272	0.0132	Ref	272,599,778	5,643	0.0207	Ref
Partially	1,190,575,743	7,129	0.0060	53.6 (51.4-	435,127,028	5,291	0.0122	28.1 (24.9-31.1)

vaccinated				55.6)				
Fully vaccinated	99,558,609	184	0.0018	88.1 (85.4-90.3)	1,194,037,275	6,971	0.0058	74.2 (72.6-75.7)
Death								
Reference period	474,761,099	6,255	0.0131	Ref	272,587,083	7,529	0.0276	Ref
Partially vaccinated	1,190,384,840	8,518	0.0072	49.3 (47.0-51.5)	434,742,763	6,988	0.0161	29.4 (26.7-32.0)
Fully vaccinated	99,567,659	249	0.0025	90.2 (88.3-91.8)	1,193,883,495	9,600	0.0080	74.0 (72.6-75.3)

Reference period: ≤13 days after the first dose; Partially vaccinated: ≥14 days after the first dose and without the second dose; Fully vaccinated: ≥14 days after the second dose. ICU denotes intensive care unit.

28

* Cox regression model adjusted for age, sex, region of residence, month of administration of first dose and municipal deprivation level.

Table S1. Crude and adjusted Vaccine effectiveness of Vaxzevria and CoronaVac in Brazil for COVID-19 infection, hospitalization, ICU admission and death.

	Vaxzevria/Fiocruz		CoronaVac/Butantan	
	CRUDE VE % (95% CI)	ADJUSTED VE % (95% CI)*	CRUDE VE % (95% CI)	ADJUSTED VE % (95% CI)*
Infection				
Reference period	—	—	—	—
Partially vaccinated	27.4 (26.5-28.2)	34.0 (33.2-34.7)	14.1 (12.9-15.3)	16.4 (15.2-17.5)
Fully vaccinated until 13 days	49.0 (47.3-50.6)	56.9 (55.3-58.5)	38.2 (37.2-39.1)	40.3 (39.4-41.2)
Fully vaccinated	63.2 (61.7-64.7)	70.0 (68.6-71.3)	52.5 (51.7-53.3)	54.2 (53.4-55.0)
Hospitalization				
Reference period	—	—	—	—
Partially vaccinated	45.3 (43.8-46.7)	52.2 (50.9-53.4)	24.1 (22.1-26.0)	26.6 (24.6-28.4)
Fully vaccinated until 13 days	53.8 (50.5-56.9)	69.6 (67.2-71.8)	55.0 (53.6-56.4)	57.3 (56.0-58.6)
Fully vaccinated	79.0 (76.5-81.2)	86.8 (85.2-88.2)	71.0 (70.0-72.0)	72.6 (71.6-73.6)
ICU admission				
Reference period	—	—	—	—
Partially vaccinated	46.5 (44.0-48.9)	54.0 (51.8-56.0)	25.3 (22.1-28.4)	28.1 (24.9-31.1)

Fully vaccinated until 13 days	51.5 (45.6-56.8)	69.2 (65.0-72.8)	55.8 (53.5-57.9)	58.1 (55.9-60.1)
Fully vaccinated	80.2 (76.0-83.7)	88.1 (85.4-90.3)	72.6 (70.9-74.2)	74.2 (72.6-75.7)
Death				
Reference period	—	—	—	—
Partially vaccinated	39.7 (37.0-42.3)	49.3 (47.0-51.5)	26.9 (24.2-29.6)	29.4 (26.7-32.0)
Fully vaccinated until 13 days	31.9 (24.9-38.3)	72.1 (69.1-74.9)	56.2 (54.3-58.1)	58.7 (56.9-60.4)
Fully vaccinated	74.8 (70.0-78.8)	90.2 (88.3-91.8)	72.1 (70.7-73.5)	74.0 (72.6-75.3)

* Cox regression model adjusted for age, sex, region of residence, month of administration of first dose and municipal deprivation level.

Table S2. Vaccine effectiveness of Vaxzevria and CoronaVac in Brazil by age groups for COVID-19 infection, hospitalization, ICU admission and death.

	Vaxzevria/Fiocruz					CoronaVac/Butantan				
	<60	**60-69**	**70-79**	**80-89**	**≥90**	**<60**	**60-69**	**70-79**	**80-89**	**≥90**
Infection										
Partially vaccinated	38.8 (37.9-39.7)	23.1 (21.3-24.9)	25.9 (20.3-31.1)	28.2 (24.5-31.7)	-43.0 (-71.2 to -19.5)	13.8 (11.6-16.0)	15.4 (13.0-17.8)	25.0 (23.1-26.9)	1.5 (-3.0 to 5.9)	-19.3 (-30.5 to -9.2)
Fully vaccinated until 13 days	54.4 (51.9-56.8)	72.2 (68.2-75.8)	60.9 (56.4-65.0)	57.9 (55.1-60.5)	21.5 (1.4-37.6)	31.1 (29.2-32.9)	38.1 (36.1-40.0)	52.5 (51.2-53.8)	37.1 (33.9-40.1)	9.1 (0.3-17.2)
Fully vaccinated	62.5 (60.2-64.7)	78.5 (73.3-82.6)	79.2 (75.7-82.2)	78.3 (76.4-80.1)	46.9 (30.9-59.3)	44.6 (43.0-46.2)	55.9 (54.3-57.4)	61.9 (60.7-63.1)	57.1 (54.7-59.5)	31.7 (24.4-38.2)
Hospitalization										
Partially vaccinated	64.1 (62.6-65.5)	44.9 (42.4-47.4)	32.9 (25.2-39.8)	32.9 (28.0-37.4)	-31.1 (-66.1 to -3.4)	33.7 (27.1-39.7)	29.5 (25.8-33.0)	32.5 (29.9-35.1)	8.2 (2.1-13.8)	-16.2 (-31.2 to -2.9)

Fully vaccinated until 13 days	83.8 (77.7-88.2)	83.3 (77.3-87.8)	71.9 (66.4-76.5)	66.6 (63.3-69.7)	34.9 (11.1-52.4)	67.1 (62.8-70.8)	60.2 (57.6-62.6)	62.2 (60.4-63.9)	42.7 (38.6-46.6)	12.4 (0.6-22.8)
Fully vaccinated	94.2 (89.8-96.6)	91.7 (84.3-95.6)	88.4 (84.6-91.2)	86.9 (84.9-88.7)	54.9 (35.4-68.5)	84.2 (81.3-86.7)	78.2 (76.3-79.8)	74.0 (72.6-75.4)	63.0 (59.9-66.0)	32.7 (22.8-41.3)
ICU admission										
Partially vaccinated	65.1 (62.5-67.6)	48.9 (44.8-52.7)	37.4 (25.1-47.7)	33.9 (25.6-41.3)	-35.4 (-110.9 to 13.1)	32.1 (19.4-42.8)	29.0 (23.1-34.5)	33.1 (28.8-37.1)	18.1 (8.6-26.6)	-27.8 (-59.6 to -2.3)
Fully vaccinated until 13 days	83.2 (70.2-90.6)	82.4 (71.2-89.3)	69.3 (59.5-76.7)	68.0 (62.3-72.8)	5.8 (-60.4 to 44.7)	69.1 (61.1 - 75.4)	61.7 (57.7-65.4)	60.9 (57.9-63.6)	46.4 (39.5-52.5)	11.3 (-12.3 to 29.9)
Fully vaccinated	95.5 (85.8-98.6)	93.2 (78.7-97.9)	87.4 (80.5-91.9)	89.3 (86.0-91.8)	39.7 (-11.7-67.5)	80.8 (74.5-85.6)	78.7 (75.8-81.3)	75.7 (73.5-77.8)	65.1 (59.9-69.7)	37.2 (18.4-51.6)
Death										
Partially vaccinated	64.8 (61.8-67.6)	45.4 (41.0-49.4)	37.1 (26.9-45.8)	38.1 (32.2-43.4)	-40.6 (-84.5 to -7.1)	41.7 (26.4-53.9)	35.7 (30.3-40.7)	38.2 (34.7-41.5)	10.1 (2.7-1.07)	-22.1 (-40.7 to -5.9)

Fully vaccinated until 13 days	80.7 (57.6-91.2)	88.5 (78.9-93.7)	77.2 (70.5-82.4)	71.3 (67.4-74.7)	45.2 (19.4-62.8)	66.1 (54.9-74.5)	64.1 (60.3-67.4)	65.5 (63.2-67.6)	46.9 (41.9-51.5)	10 (-4.4 to 22.4)
Fully vaccinated	93.3 (72.1-98.4)	89.6 (71.8-96.2)	92.5 (88.1-95.3)	91.2 (89.1-92.9)	70.5 (51.4-82.1)	76.5 (66.9-83.3)	78.7 (76.6-80.0)	78.3 (76.6-80.0)	67.3 (63.6-70.6)	35.4 (23.8-45.1)

*Obtained through Cox regression model adjusted for age, sex, region of residence, month of administration of first dose and municipal deprivation level

Table S3. Robustness analysis with different time windows as reference period

	Vaxzevria/Fiocruz VE % (95% CI)	CoronaVac/Butantan VE % (95% CI)
Reference Period:	0-10 days	0-10 days
Infection		
Partially vaccinated	33.2 (32.3-34.0)	16.5 (15.2-17.8)
Fully vaccinated until 13 days	55.5 (53.7-57.3)	38.0 (36.9-39.0)
Fully vaccinated	69.8 (68.2-71.3)	54.6 (53.7-55.5)
Hospitalization		
Partially vaccinated	51.3 (49.9-52.7)	25.5 (23.4-27.6)
Fully vaccinated until 13 days	67.6 (64.8-70.1)	55.4 (53.8-56.8)
Fully vaccinated	86.0 (84.1-87.6)	72.5 (71.4-73.6)
ICU admission		
Partially vaccinated	53.7 (51.3-56.0)	27.8 (24.3-31.1)
Fully vaccinated until 13 days	67.2 (62.4-71.3)	56.7 (54.2-59.0)
Fully vaccinated	87.4 (84.3-89.9)	74.1 (72.3-75.8)
Death		
Partially vaccinated	48.2 (45.6-50.6)	28.8 (25.8-31.6)
Fully vaccinated until 13 days	70.4 (66.8-73.7)	57.9 (55.8-59.9)
Fully vaccinated	89.2 (86.9-91.1)	73.7 (72.1-75.2)

Table S4: Percentage of events with laboratory confirmation and VE using all cases (laboratory and clinical suspected)

	Vaxzevria/Fiocruz				Coronavac/Butantan			
	Events-Laboratory Confirmed	Events-Confirmed or Clinical Suspected	% Confirmed	VE* (95% CI)	**Events-Laboratory Confirmed**	Events-Confirmed or Clinical Suspected	% Confirmed	VE* (95% CI)
Hospitalization								
Reference period	18.420	23.368	78.8	Ref	15.080	19.672	76.6	Ref
Partially vaccinated	20,998	27,946	75.1	50.7 (49.6-51.9)	14,484	19,182	75.5	25.5 (23.8-27.2)
Fully vaccinated	574	845	67.9	85.8 (84.3-87.1)	20,299	26,836	75.6	71.5 (70.6-72.4)
ICU admission								
Reference period	6.272	7,693	81.5	Ref	5,643	7,176	78.6	Ref
Partially vaccinated	7,129	9,164	77.8	52.4 (50.5-54.3)	5,291	6,875	77.0	26.9 (24.1-29.6)
Fully vaccinated	184	262	70.2	87.5 (85.1-89.5)	6.971	9,015	77.3%	73.2 (71.8-74.6)

36

Death								
Reference period	6,255	7,749	80.7	Ref	7,529	9,608	78.4	Ref
Partially vaccinated	8,518	11,091	76.8	47.8 (45.7-49.8)	6,988	9,043	77.3	28.7 (26.3-31.0)
Fully vaccinated	249	359	69.4	89.5 (87.8-91.0)	9,600	12,262	78.2	73.4 (72.2-74.6)

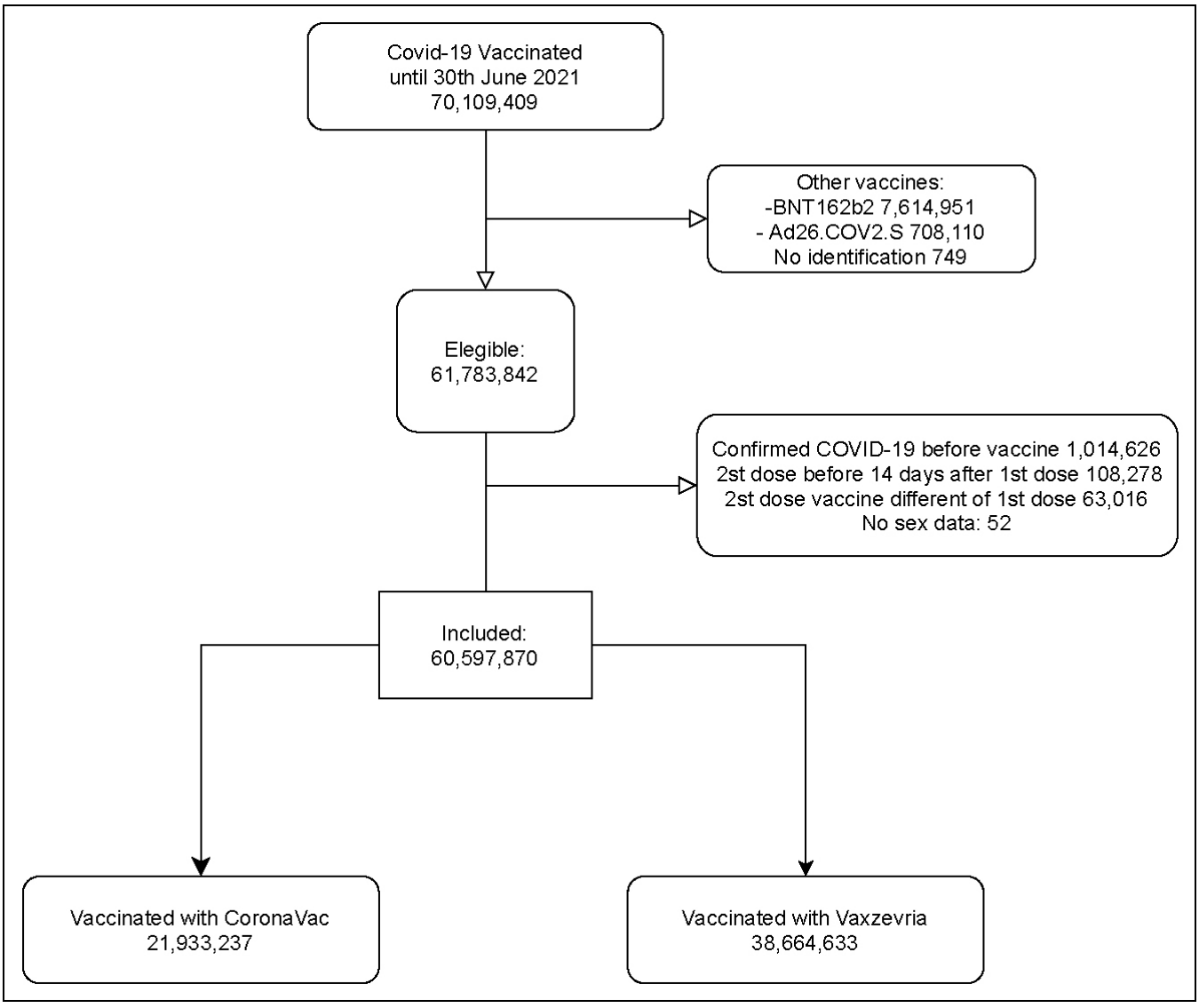
*Obtained through Cox regression model adjusted for age, sex, region of residence, month of administration of first dose and municipal deprivation level

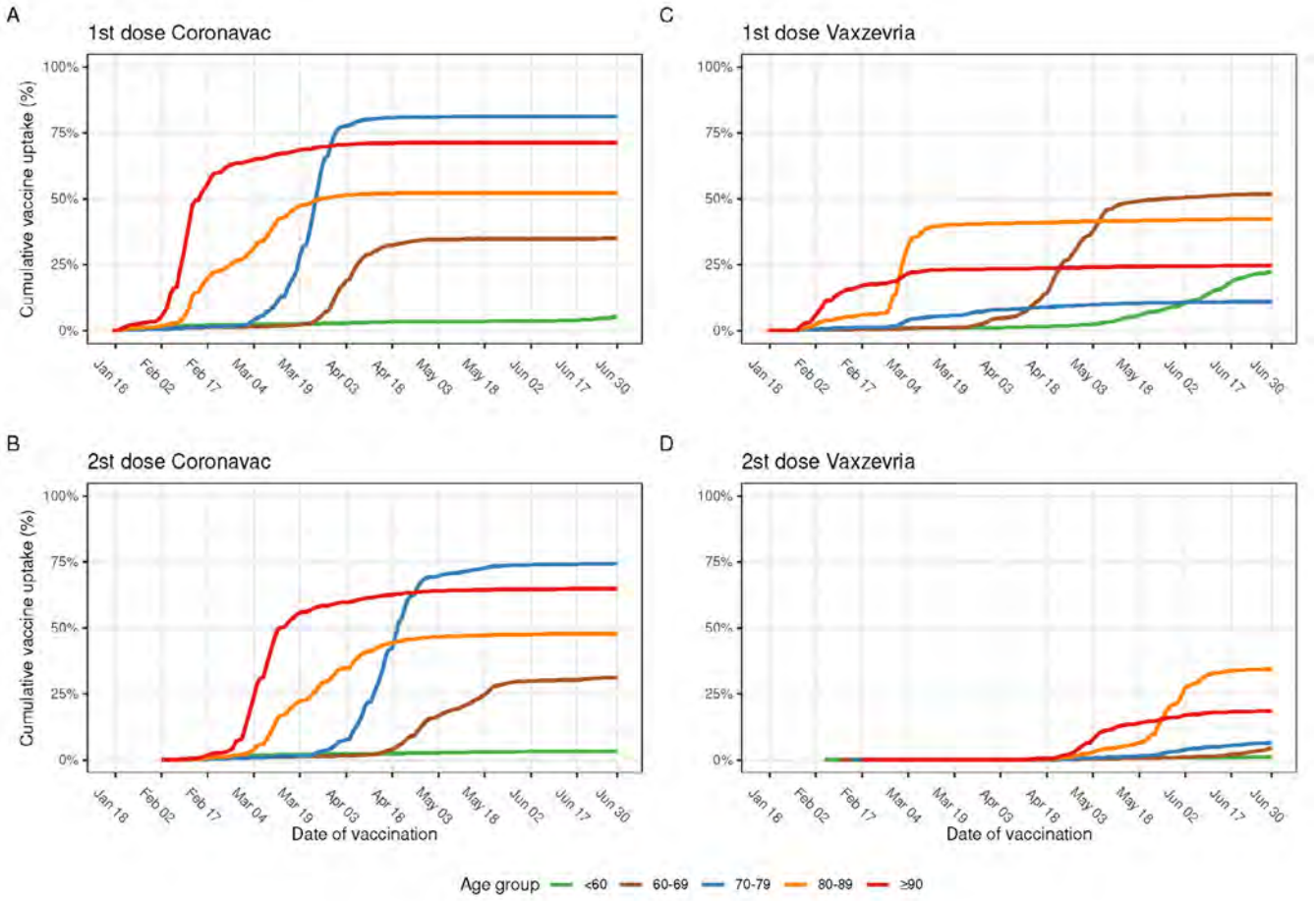
Figures legends

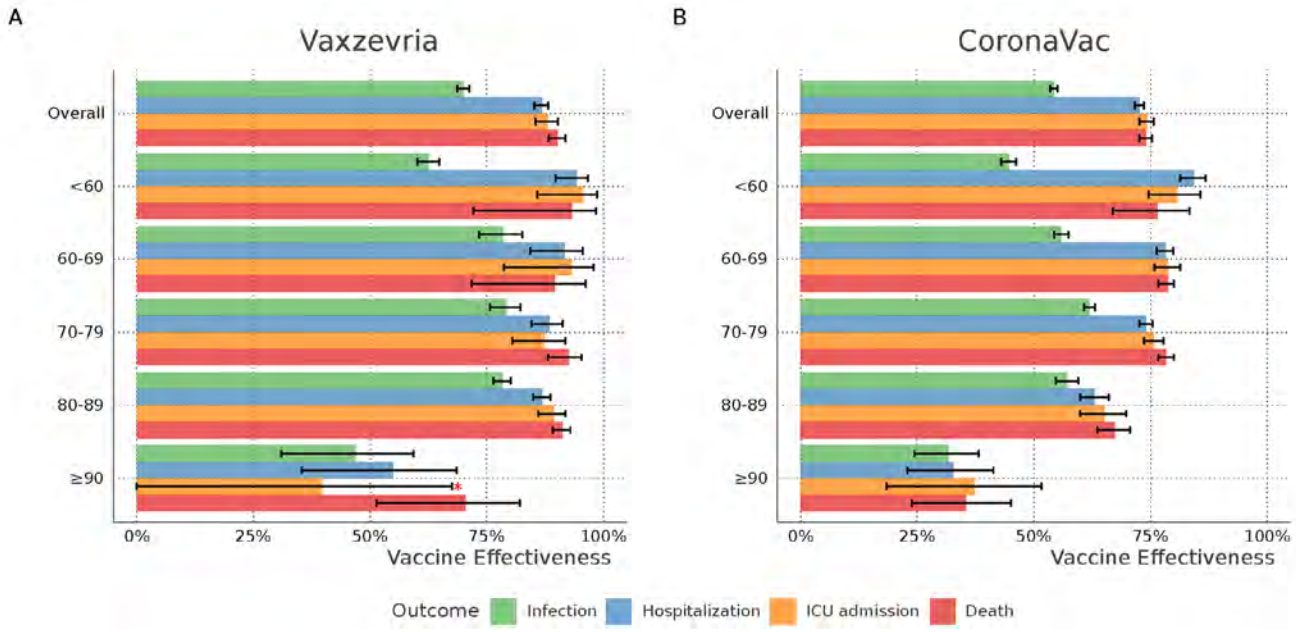
Figure 1. Flowchart of the selection of the study individuals vaccinated between 18th January and 30 June 2021. Eligible participants received at least one dose of CoronaVac or Vaxzevria vaccine between January 18 and June 30, 2021. We excluded persons with confirmed COVID-19 diagnosis in 2021 before the first dose and all persons with different vaccines from CoronaVac or Vaxzevria

Figure 2. Coverage of first and second dose of CoronaVac and Vaxzevria in Brazil during the study period. The panels A, B, C and D shown the rate and coverage of the vaccination program regarding CoronaVac and Vaxzevria, A and C regarding first dose between January 18 and June 30 and panels B and D the second dose until 30 June 2021.

Figure 3. Vaccine effectiveness of Vaxzevria and CoronaVac in Brazil by age group. VE (1-Hazard Ratio) was obtained through Cox regression adjusted for age, sex, region of residence, the month of administration of first dose, and municipal deprivation level (IBP). *The point estimate and confidence interval for ICU admission in ≥90 y.o. are 39.7 (95%CI -11.7 to 67.5%), the large confidence interval is reflect of the small sample size and number of events in this group, 35 in the reference period and 33 in the fully vaccinated.


Covid-19 Vaccinated
until 30th June 2021
70,109,409
Other vaccines:
-BNT162b2 7,614,951
- Ad26.COV2.S 708,110
No identification 749
Elegible:
61,783,842
Confirmed COVID-19 before vaccine 1,014,626
2st dose before 14 days after 1st dose 108,278
2st dose vaccine different of 1st dose 63,016
No sex data: 52
Included:
60,597,870
Vaccinated with CoronaVac
21,933,237
Vaccinated with Vaxzevria
38,664,633


A
1st dose Coronavac
C
1st dose Vaxzevria
B
2st dose Coronavac
D
2st dose Vaxzevria
Cumulative vaccine uptake (%)
100%
75%
50%
25%
0%
Jan 18
Feb 02
Feb 17
Mar 04
Mar 19
Apr 03
Apr 18
May 03
May 18
Jun 02
Jun 17
Jun 30
Date of vaccination
Age group
<60
60-69
70-79
80-89
≥90


A
Vaxzevria
B
CoronaVac
Overall
<60
60-69
70-79
80-89
≥90
0%
25%
50%
75%
100%
Vaccine Effectiveness
Outcome
Infection
Hospitalization
ICU admission
Death

5.10. Estudo atesta a eficácia da CoronaVac contra a variante gama (P.1) entre idosos

Uma pesquisa publicada na plataforma de preprints MedRxiv atesta a eficácia da Coronavac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, na prevenção da variante gama (P.1, amazônica) do vírus SARS-CoV-2 em idosos com mais de 70 anos.

A eficácia da vacina contra hospitalizações 14 dias após a aplicação da segunda dose foi de 59%, e contra mortes, de 71,4%. O indicador variou com o aumento de idade: entre os indivíduos com idade de 70 a 74 anos, a eficácia foi de 61,8% contra a doença sintomática, de 80,1% contra hospitalizações e de 86% contra mortes.

"Em resumo, ficou evidenciado que um esquema de duas doses de CoronaVac foi eficaz na prevenção de casos sintomáticos de Covid-19 e na prevenção de desfechos clínicos mais graves entre idosos frente à variante gama", afirmam os autores no artigo.

O trabalho foi realizado por pesquisadores ligados à Secretaria de Saúde do Estado de São Paulo, à Organização Pan-Americana de Saúde, à Universidade de São Paulo e às universidades norte-americanas da Flórida e de Yale, entre outras instituições. Foram investigados 43.774 adultos com 70 anos ou mais, residentes no estado de São Paulo, todos sintomáticos para Covid-19.

O objetivo da pesquisa era estimar a eficácia da CoronaVac contra a Covid-19 sintomática na população idosa do estado de São Paulo durante a ampla circulação da variante gama, entre janeiro e abril de 2021.

Os autores concluem que, embora outras pesquisas ainda devam contribuir para reafirmar a eficácia do CoronaVac contra a variante gama, os resultados fornecem evidências que suportam o uso da vacina no Brasil e nos demais países da América do Sul que enfrentam a disseminação da variante gama do SARS-CoV-2.

Publicado em: 21/07/2021

Effectiveness of the CoronaVac vaccine in the elderly population during a Gamma variant-associated epidemic of COVID-19 in Brazil: A test-negative case-control study

Otavio T. Ranzani[1,2]*, Matt D.T. Hitchings[3,4]*, Murilo Dorion[5], Tatiana Lang D'Agostini[6], Regiane Cardoso de Paula[6], Olivia Ferreira Pereira de Paula[6], Edlaine Faria de Moura Villela[6], Mario Sergio Scaramuzzini Torres[7], Silvano Barbosa de Oliveira[8,9], Wade Schulz[10], Maria Almiron[8], Rodrigo Said[8], Roberto Dias de Oliveira[11], Patricia Vieira da Silva[12], Wildo Navegantes de Araújo[8,9,13], Jean Carlo Gorinchteyn[14], Jason R. Andrews†[15], Derek A.T. Cummings†[3,4], Albert I. Ko†[5,16], Julio Croda†[5,12,17]

1-Barcelona Institute for Global Health, ISGlobal, Barcelona, Spain
2-Pulmonary Division, Heart Institute (InCor), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brazil
3-Department of Biology, University of Florida, Gainesville, FL, USA
4-Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA
5-Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, USA
6-Disease Control Coordination of the São Paulo State Department of Health, São Paulo, Brazil
7-Municipal Health Secretary of Manaus, Brazil, AM, Brazil
8-Pan American Health Organization, Brasília, DF, Brazil
9-Universidade de Brasília, Brasília, DF, Brazil
10-Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT, USA
11-State University of Mato Grosso do Sul - UEMS, Dourados, MS, Brazil
12-Universidade Federal de Mato Grosso do Sul - UFMS, Campo Grande, MS, Brazil
13-National Institute for Science and Technology for Health Technology Assessment, Porto Alegre, RS, Brazil
14-Health Secretariat of the State of São Paulo, São Paulo, Brazil
15-Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, CA, USA
16-Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, BA, Brazil
17-Fiocruz Mato Grosso do Sul, Fundação Oswaldo Cruz, Campo Grande, MS, Brazil

*Authors contributed equally
†Authors contributed equally

Correspondence to: Prof Julio Croda, Universidade Federal de Mato Grosso do Sul and Fundação Oswaldo Cruz, julio.croda@fiocruz.br

Keywords
COVID-19; CoronaVac; inactivated whole-virus vaccine, Gamma variant; test-negative study; case-control study; Brazil

Word count: 3,840

ABSTRACT

Objective To estimate the effectiveness of the inactivated whole-virus vaccine, CoronaVac, against symptomatic COVID-19 in the elderly population of São Paulo State, Brazil during widespread circulation of the Gamma variant.

Design Test negative case-control study.

Setting Health-care facilities in São Paulo State, Brazil.

Participants 43,774 adults aged 70 years or older who were residents of São Paulo State and underwent SARS-CoV-2 RT-PCR testing from January 17 to April 29, 2021. 26,433 cases with symptomatic COVID-19 and 17,622 symptomatic, test negative controls were selected into 7,950 matched pairs, according to age, sex, self-reported race, municipality of residence, prior COVID-19 status and date of RT-PCR testing.

Intervention Vaccination with a two-dose regimen of CoronaVac.

Main outcome measures RT-PCR confirmed symptomatic COVID-19 and COVID-19 associated hospitalizations and deaths.

Results Adjusted vaccine effectiveness against symptomatic COVID-19 was 18.2% (95% CI, 0.0 to 33.2) in the period 0-13 days after the second dose and 41.6% (95% CI, 26.9 to 53.3) in the period ≥14 days after the second dose. Adjusted vaccine effectiveness against hospitalisations was 59.0% (95% CI, 44.2 to 69.8) and against deaths was 71.4% (95% CI, 53.7 to 82.3) in the period ≥14 days after the second dose. Vaccine effectiveness ≥14 days after the second dose declined with increasing age for the three outcomes, and among individuals aged 70-74 years it was 61.8% (95% CI, 34.8 to 77.7) against symptomatic disease, 80.1% (95% CI, 55.7 to 91.0) against hospitalisations and 86.0% (95% CI, 50.4 to 96.1) against deaths.

Conclusions Vaccination with CoronaVac was associated with a reduction in symptomatic COVID-19, hospitalisations and deaths in adults aged 70 years or older in a setting with extensive Gamma variant transmission. However, significant protection was not observed until completion of the two-dose regimen, and vaccine effectiveness declined with increasing age amongst this elderly population.

Summary boxes

What is already known on this topic

- Randomised controlled trials (RCT) have yielded varying estimates (51 to 84%) for the effectiveness of the inactivated whole-virus vaccine, CoronaVac, against symptomatic COVID-19.
- Current evidence is limited on whether CoronaVac is effective against severe disease or death caused by the SARS-CoV-2 variant of concern, Gamma, or in the setting of extensive Gamma variant circulation.
- More evidence is needed for the real-world effectiveness of CoronaVac and other inactivated vaccines among elderly individuals, a population that was underrepresented in RCTs of these vaccines.

What this study adds

- A two-dose regimen of CoronaVac provides significant protection against symptomatic COVID-19, hospitalisations and deaths among adults ≥70 years of age in the setting of widespread Gamma variant transmission.
- Significant protection did not occur until ≥14 days after administration of the second dose of CoronaVac.
- The effectiveness of CoronaVac declines with increasing age in the elderly population.

Introduction

The coronavirus disease (COVID-19) pandemic has caused 3.9 million deaths worldwide as of early July 2021,[1] and has imparted disproportionately high mortality and morbidity on the elderly.[2] A key question is whether the authorised COVID-19 vaccines are effective in the elderly, who may have impaired immune responses[3,4] and are underrepresented in randomised controlled trials (RCTs).[5–7] mRNA and adenovirus vector-based vaccines have been shown to be effective against COVID-19 in elderly individuals,[8,9] but evidence is limited for the effectiveness of inactivated vaccines in these populations.[7,10–12]

CoronaVac, an inactivated whole-virus vaccine, has been approved by 32 countries and jurisdictions,[10] and has been implemented as part of mass vaccination campaigns in low-income and middle-income countries, many of which are experiencing COVID-19 epidemics due to the emergence of SARS-CoV-2 variants of concern (VOC). RCTs of a two-dose CoronaVac regimen in healthcare workers and the general population have yielded varying estimates (51 to 84%) of vaccine efficacy against symptomatic COVID-19.[5,7,10] The World Health Organisation (WHO) Emergency Use Listing (EUL) procedure approved CoronaVac in early June 2021, but identified an evidence gap for the effectiveness of this vaccine in adults aged 60 and above.[11] The WHO EUL cited an observational study in Chile,[10,12] which found that the adjusted effectiveness of CoronaVac, starting 14 days after the second dose, was 66.6% among adults aged 60 years and older. During the study period, the variant of concern (VOC) Gamma was detected in 28.6% of SARS-CoV-2 genomes.[12] Furthermore, evidence from RCTs or observational studies have not

addressed whether CoronaVac provides significant protection after administration of the first vaccine dose or in the setting of widespread VOC transmission.[5,10,11]

Brazil has experienced one of the world's highest COVID-19 burdens during the pandemic with more than 18 million cases and 526,000 deaths as of early July 2021.[1,13] VOCs, and in particular the Gamma variant, have played an important role in the recent epidemic wave in Brazil which began in early 2021.[14–16] The Gamma variant, which was first detected in Manaus, has increased transmissibility,[16] has accrued mutations associated with decreased *in vitro* seroneutralisation,[17–19] and at present, accounts for the majority of SARS-CoV-2 isolates genotyped in Brazil from 1 January 2021.[14,20] In the setting of a large Gamma variant-associated epidemic in São Paulo, the most populous state in Brazil, we conducted a matched, test-negative,[21] case-control study to evaluate the real-world effectiveness of CoronaVac against symptomatic COVID-19 and severe clinical outcomes in the elderly population.

Methods

Study setting

The State of São Paulo (23°3'S, 46°4'W) has 645 municipalities and 46 million inhabitants, among which 3.23 million are ≥70 years of age.[22] The state experienced three successive COVID-19 epidemic waves during which 2,997,282 cases (cumulative incidence rate: 6,475 per 100,000 population) and 100,649 deaths (cumulative mortality: 217 per 100,000 population) have been reported as of 9 May 2021 (Figure 1A, Supplementary Figure 1).[23] The State Secretary of Health of Sao Paulo (SES-SP) initiated a COVID-19 vaccination campaign for the

general population on 17 January 2021 according to an age-based prioritisation strategy (Figure 1, B-D) and is administering a two-dose regimen of CoronaVac, separated by a two to four week interval, and a two-dose regimen of ChAdOx1, separated by a 12 week interval.[24] As of 29 April 2021, 8.63 million doses (5.16 first and 3.47 second million doses) have been administered of CoronaVac and 2.06 million doses (1.987 first and 0.07 second million doses) of ChAdOx1.

Study design

We conducted a matched test-negative case-control study to estimate the effectiveness of CoronaVac in reducing the odds of symptomatic RT-PCR-confirmed COVID-19 in adults ≥70 years of age from São Paulo State during the period from 17 January 2021, the start of COVID-19 vaccination, to 29 April 2021. Test-negative design studies have provided estimates of vaccine effectiveness in concordance with those obtained from RCTs[25,26] and have been used extensively to evaluate vaccines against respiratory infections,[27] including COVID-19.[8,21] We chose the test-negative design because of the feasibility of accessing information on individuals who received SARS-CoV-2 testing from São Paulo State surveillance systems and the opportunity to control for potential biases, such as healthcare-seeking behaviour and access to testing.[21] The study population was adults ≥70 years of age who had a residential address in São Paulo State, underwent SARS-CoV-2 RT-PCR testing during the study period, and had complete and consistent information between data sources on age, sex, residence, and vaccination and testing status and dates. We matched symptomatic test-negative controls to COVID-19 cases by date of testing to address potential sources of bias that may vary during the course of an

epidemic, as well as by participant characteristics of age, gender, self-reported race, municipality of residence, and prior COVID-19 status.

The study design and statistical analysis plan were specified in advance of extracting information from data sources and are described in a publicly available protocol (https://github.com/juliocroda/VebraCOVID-19) and the Supplement. In the protocol, we pre-specified power thresholds for conducting analyses on the effectiveness of CoronaVac and ChAdOx1. These thresholds were achieved for CoronaVac but not for ChAdOx1 because of lower rates of ChAdOx1 administration in the population. We therefore restricted the evaluation of vaccine effectiveness to CoronaVac. The study was approved by the Ethical Committee for Research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021).

Data Sources

We obtained individual-level information on demographic characteristics, comorbidities, SARS-CoV-2 testing, and COVID-19 vaccination during the study period by extracting information on 6 May 2021 from the SES-SP laboratory testing registry (GAL), the national surveillance databases for COVID-19-like illnesses (e-SUS) and severe acute respiratory illness (SIVEP-Gripe), and the SES-SP vaccination registry (Vacina Já). Notification of suspected COVID-19 cases and SARS-CoV-2 testing results is compulsory in Brazil. The information technology bureau of the São Paulo State Government (PRODESP) linked individual-level records from the four databases using CPF numbers (Brazilian citizens' unique identifier code) and provided anonymised datasets. We

retrieved information on SARS-CoV-2 variants from genotyped isolates deposited in the GISAID database.[20]

Selection of cases and matched controls

Cases were selected from the study population who had symptomatic COVID-19, defined as an individual who had a COVID-19-like illness; had a positive SARS-CoV-2 RT-PCR test result from a respiratory sample which was collected within 10 days after the onset of symptoms; and did not have a positive RT-PCR test in the preceding 90-day period. Controls were selected from the study population who had a COVID-19-like illness; had a negative SARS-CoV-2 RT-PCR test result from a respiratory sample that was collected within 10 days after the onset of symptoms;[21] and did not have a positive RT-PCR test in the prior 90 days during the study period or in the subsequent 14 days. Cases and controls were excluded if they received the ChAdOx1 vaccine before sample collection for RT-PCR testing. COVID-19-like illness was defined as the presence of one or more reported COVID-19 related symptoms.[28]

We matched one test-negative control to each case according to RT-PCR sample collection date (±3 days); age category (5-year age bands, e.g, 70-74, 75-79 years); municipality of residence; self-reported race (defined as brown, black, yellow, white, or indigenous);[29] and previous symptomatic events that were reported to the surveillance systems[28] between February 1, 2020 and January 16, 2021, as a proxy for previous COVID-19 infection. Matching factors were chosen from variables that were associated with vaccination coverage or timing, and with SARS-

CoV-2 infection risk or healthcare access (see protocol in Supplement).[21] Upon identification of each case, a single control was randomly chosen from the set of all eligible matching controls.

Statistical analysis

We estimated the effectiveness of CoronaVac against symptomatic COVID-19 during the periods 0-13 and ≥14 days after the second vaccine dose and ≥14 days after a single vaccine dose. Furthermore, we estimated the effectiveness of a single dose during the period 0-13 days after the first dose, when the vaccine has no or limited effectiveness.[5,30,31] An association during this period may serve as an indicator of unmeasured confounding in the effectiveness estimate.[32] The reference group for vaccination status was individuals who had not received a first vaccine dose before the date of sample collection.

We used conditional logistic regression to estimate the odds ratio (OR) of vaccination among cases and controls. 1-OR provided an estimate of vaccine effectiveness under the assumptions of a test-negative design.[33] We included age and COVID-19-associated comorbidities (cardiovascular, renal, neurological, haematological, or hepatic comorbidities, diabetes, chronic respiratory disorder, obesity, or immunosuppression) as covariates in the model. We evaluated nonlinearity for age using restricted cubic splines and chose the parsimonious model comparing nested models with a likelihood ratio test. Furthermore, we conducted a *post hoc* sensitivity analysis that incorporated the calendar date of RT-PCR sample collection in the model to evaluate potential residual confounding that may not be addressed by the matching criteria

We estimated the vaccine effectiveness against acute respiratory illness (ARI) associated hospitalizations and deaths in a *post hoc* analysis. In separate analyses, we selected matched pairs in which the case had the secondary outcome of interest.[34,35] We fit the same conditional logistic regression model as for the primary outcome.

We conducted a pre-specified analysis of vaccine effectiveness among age sub-groups for the primary and secondary outcomes, but could not perform analyses stratified by previous COVID-19 documented infection because of small numbers. Additional *post hoc* analyses were performed of vaccine effectiveness for the primary outcome for subgroups stratified by sex, number of chronic comorbidities (none vs. at least one), the two most frequent chronic comorbidities (cardiovascular disease and diabetes), and region of residence ("Grande São Paulo" health region vs. others). Interaction terms were incorporated into the model to evaluate the association of each subgroup of interest with vaccine effectiveness ≥14 days after the second dose.

Power calculation

Our protocol specified that we would conduct proposed analyses after achieving ≥80% power to identify a vaccine effectiveness of 40% against symptomatic COVID-19 for the comparison of ≥14 days after the second dose of CoronaVac and not receiving a vaccine dose. The power was simulated fitting conditional logistic regressions on 1,000 simulated datasets. After extracting the surveillance databases on May 6, 2021 and generating matched case-control pairs, we determined that the power of the study was 99.9% and proceeded to conduct the pre-specified

analyses. We did not perform an analysis for ChAdOx1 since the simulated power was 31% to identify a vaccine effectiveness of 40% for the comparison of ≥28 days after the first dose of ChAdOx1 and not receiving a vaccine dose. All analyses were done in R, version 4.0.2.

Results

COVID-19 epidemic and vaccination campaign in São Paulo State

São Paulo State experienced three COVID-19 epidemic waves during which peak incidence occurred in July 2020 for the first wave (Supplementary Figure 1), January 2021 for the second wave and March 2021 for the third wave (Figure 1A). The second wave was preceded in November 2020 by an increase in the prevalence of the Zeta variant among genotyped isolates from São Paulo State deposited into the GISAID database (Figure 1E). The third wave was preceded in January 2021 by an increase in the prevalence of the Gamma variant among genotyped isolates. The Gamma variant replaced other SARS-CoV-2 variants[20] and accounted for 79% (3,834/4,887) of the genotyped isolates that were reported in GISAID during the study period and 86% (3,584/4,192) of genotyped isolates that were reported between 1 March to 29 April 2021 when the majority of discordant case-control pairs were identified (Supplementary Figure 2). The vaccination campaign, initiated on January 17, 2021, achieved an estimated coverage of roughly 85% for the first (2.82 million) and 65% for second (2.10 million) CoronaVac doses among adults ≥70 years of age by April 29, 2021 (Figure 1B-D). After initiation of the vaccination campaign and during the third epidemic wave, COVID-19 incidence increased and peaked in late March in all age groups except for adults ≥90 years of age (Figure 1A).

Study population

Among 43,774 individuals eligible for study inclusion (Figure 2), 15,852 (36.2%) who provided 15,900 RT-PCR test results were selected into 7,950 matched case and control pairs. There were 38 individuals that contributed two times as controls and 10 individuals one time as control and one time as case. Table 1 shows the characteristics of eligible individuals with positive and negative RT-PCR tests and selected cases and matched controls. A higher proportion of cases had reported comorbidities than controls. Supplementary Table 1 shows the distribution of matched pairs according to the vaccination status of cases and controls at the time of RT-PCR testing. The majority of discordant pairs, based on vaccination status, were selected after 14 March 2021 (Supplementary Figure 3). Cases and controls who completed the two dose vaccine regimen had similar inter-dose intervals (mean 29 vs. 25 days). Likewise, cases and controls who were vaccinated had similar distributions for the intervals between administration of vaccine doses and RT-PCR testing (Table 1 and Supplementary Figure 3). The characteristics of the matched case and control pairs which were selected for the analysis of secondary outcomes of hospitalisation (n=8,078) and death (n=4,104) are shown in Supplementary Tables 2 and 3.

Vaccine effectiveness

The adjusted effectiveness of the two-dose CoronaVac schedule against symptomatic COVID-19 was 18.2% (95% CI 0.0 to 33.2) in the period 0-13 days and 41.6% (95% CI 26.9 to 53.3) in the period ≥14 days after administration of the second dose (Table 2). We did not identify a significant reduction or increase in the odds of COVID-19 in the time periods following a single vaccine dose, including the period 0-13 days which serves as a potential bias-indicator.

Increasing number of comorbidities was significantly associated with increased odds of COVID-19. In a sensitivity analysis including calendar date of testing as a covariate, vaccine effectiveness was 19.3% (95% CI 1.3 to 34) in the period 0-13 day and 42.3% (95% CI 27.7 to 53.9) in the period ≥14 days after administration of the second dose.

In the period starting 14 days after the second dose, the adjusted effectiveness of the two-dose schedule was 59.0% (95% CI 44.2 to 69.8) against hospitalisation and 71.4% (95% CI 53.7 to 82.3) against deaths (Table 2). In general, statistically significant protection was not observed until after the second dose, and the vaccine effectiveness in the "bias-indicator" period 0-13 days after the first dose was low.

Vaccine effectiveness against symptomatic COVID-19 in the period ≥14 days after the second dose declined with increasing age and was 61.8% (95% CI 34.8 to 77.7) among individuals 70-74 years old, 48.9% (95% CI 23.3 to 66.0) among 75-79 years old, and 28.0% (95% CI 0.6 to 47.9) among individuals ≥80 years of age ($p_{interaction}$ = 0.05)(Figure 3). The same pattern was observed for hospitalisations ($p_{interaction}$ = 0.04) and deaths ($p_{interaction}$ = 0.19), yielding effectiveness of 80.1% (95% CI 55.7 to 91.0) for hospitalisations and 86.0% (95% CI 34.8 to 77.7) for deaths among the 70-74 years age group (Figure 3 and Supplementary Table 4).

Vaccine effectiveness against symptomatic COVID-19 disease did not differ among sub-groups defined by sex, presence of comorbidities, reported cardiovascular disease, or regions of residence. However, individuals with reported diabetes had lower protection than those

without reported diabetes (VE 26.9% vs. 45.6% , $p_{interaction}$ = 0.12) during the period starting 14 days after the 2nd dose (Supplementary Table 5 and Supplementary Figure 4).

Discussion

This test-negative case-control study found that a two-dose schedule of CoronaVac had a real-world effectiveness of 41.6% (95% CI 26.9 to 53.3) against symptomatic COVID-19, 59.0% (95% CI 44.2 to 69.8) against COVID associated hospitalisations, and 71.4% (95% CI 53.7 to 82.3%) against COVID-19 associated deaths among those ≥70 years during a Gamma variant-associated epidemic in Brazil. Furthermore, we have addressed several evidence gaps for the use of this vaccine: 1) vaccination with CoronaVac demonstrated an effectiveness against COVID-19, including associated severe outcomes, in the setting of widespread Gamma transmission which was similar to that found in the Brazilian RCT conducted prior to the emergence of Gamma,[5] 2) the vaccine did not confer significant protection until 14 days after completion of the two dose regimen; and 3) vaccine effectiveness declined with increasing age among adults ≥70 years of age.

Research in context

A key evidence gap, as raised in the WHO EUL for Coronavac,[11] has been the effectiveness of this vaccine in the elderly population, since this age group was not represented in the Brazilian and Turkish RCTs.[5,7,10,11] We found that CoronaVac had an effectiveness in the elderly population that was similar to that observed in RCTs of younger populations and similar to estimates of vaccine effectiveness in adults ≥60 years of age from a retrospective cohort study

in Chile.[10,12] However, we observed a significant decline in vaccine effectiveness against symptomatic COVID-19 with increasing age from 61.8% (95% CI 34.8 to 77.7) in adults 70-74 year olds to 28.0% (95% CI 0.6 to 47.9) in adults ≥80 years of age. These findings parallel real-world evidence for the BNT162b2 mRNA vaccine, which found reduced effectiveness in residents of long-term care facilities in Denmark,[36] skilled nursing facilities in the USA,[37] and the general population with ≥70 years in Finland[38] and ≥80 years of age in Israel.[39] As well as a slower immune response and lower peak of neutralising antibodies than younger populations, elderly individuals seem to have faster decay of antibodies titers.[4] Together, these findings suggest that effective COVID-19 vaccination of the very elderly (≥80 years) population may require specific vaccines or vaccination schemes.

Vaccine effectiveness was greater against severe outcomes than against symptomatic COVID-19 in all age subgroups among the elderly. This finding, consistent with RCTs and observational studies for multiple COVID-19 vaccines and across settings,[5,6,9,10,12] suggests that vaccination will reduce morbidity and mortality even if effectiveness at preventing infections is reduced among the elderly. The direct comparison of the effectiveness against hospitalisation with other vaccines and between countries is not straightforward, because hospitalisation is dependent on admission triage policies that change according to age and hospital bed availability. Therefore, a patient above 80 years with symptomatic COVID-19 has higher likelihood of being admitted compared to younger patients even if not severe, and this likelihood varies between public and private facilities and whether the health system is overwhelmed.[13] Thus, we cannot generalise our findings for protection against hospitalisations without considering this context. We

evaluated vaccine effectiveness at the individual level, not accounting for the indirect effect and the total effect from the vaccination campaign. A preliminary aggregated analysis using weekly times series of COVID-19 deaths in Brazil found a relative decrease in mortality among those ≥70 years compared with all ages after the vaccination with CoronaVac and ChAdOx1,[40] suggesting a discernible impact of vaccination on mortality at the population level. Additional investigation is required to address the duration of protection conferred by Coronavac.[7,19,21]

The absence of demonstrable effectiveness of CoronaVac until completion of the two dose regimen has profound implications for its use in an epidemic response. In contrast to COVID-19 vaccines that confer protection after the first dose,[9,41] we did not detect significant effectiveness for CoronaVac until ≥14 days after the second dose (more than six weeks after the first dose).[19] Our findings suggest that in countries where CoronaVac supplies are constrained and are experiencing high SARS-CoV-2 transmission, vaccination should prioritise completion of the two-dose regimen among the highest risk populations and avoid expanding to broader segments for which provisions for a second dose have not been secured.

Our study did not directly address the question whether vaccination with CoronaVac was effective against Gamma-variant-associated COVID-19 since we have no data on whether the analysed cases were due to Gamma variant. However, 90% (1,790/1,999) of the discordant pairs in this matched case-control study were selected during the period 1 March to 29 April 2021, when Gamma accounted for 85% of the genotyped isolates during surveillance in São Paulo state. A test-negative study in Canada evaluated ≥70 years individuals and estimated an

adjusted vaccine effectiveness of single-dose mRNA vaccines of 61% (95% CI 45-72) against the VOC Gamma compared to 72% (95% CI 58-81) for non-VOC.[42] Although further studies are required to determine the effectiveness of CoronaVac against Gamma and additional VOCs, our findings provide supportive evidence for the use of CoronaVac in countries in South America which are experiencing epidemics due to extensive spread of Gamma[20] and are administering mass vaccination with CoronaVac as part of the epidemic response.

Strengths and limitations of this study

This study has several strengths which include the large sample size and geospatial coverage, comprising the state of São Paulo with 46 million inhabitants distributed across 645 municipalities. We implemented a pre-specified publicly-available protocol, which is in accordance with the recent WHO guideline for COVID-19 vaccine effectiveness evaluation.[21] Using a test-negative design, we have addressed biases that affect observational vaccine effectiveness studies, such as health-seeking behaviour and access. Additionally, after matching and adjustment, the "bias-indicator" association between recent vaccination with a single dose 0-13 days before sample collection was close to null, suggesting that vaccinated and unvaccinated individuals did not differ in their underlying risk of testing positive for SARS-CoV-2.[8,32,43]

Our study had limitations. We could not assess the influence of a previous SARS-CoV-2 infection on vaccine effectiveness since passive surveillance identified few individuals with a positive RT-PCR or rapid antigen test before the study period. Prior to the start of the vaccination campaign, the estimated seroprevalence of COVID-19 in inhabitants who were ≥60 years of age

in the capital of São Paulo State was 19.9% (95% CI, 14.9-29.9) in January 2021.[44] Our estimates of vaccine effectiveness may therefore be subject to downward bias as unvaccinated individuals were at lower risk of reinfection. We attempted to exclude false-negative RT-PCR tests by excluding as controls patients with a subsequent positive test within 14 days after the initial testing and including only tests performed 10 days of symptom onset.[21] In addition, we restricted our study population to elderly individuals because they were a priority group for vaccination and received the large majority of CoronaVac doses during the initial stages of the campaign in Brazil; as a result, a direct comparison of the effectiveness of CoronaVac between older and younger populations was not possible. Our analyses were also limited by the lack of more refined covariates, such as frailty and chronic illness status, which could influence vaccine effectiveness in the very elderly and would not be addressed by age and reported comorbidities *per se*. Finally, although we matched for calendar time of SARS-CoV-2 testing (±3 days),[21] we cannot exclude the possibility of time-varying changes in behaviour or testing practices among participants that were not addressed by our matching criteria and may introduce bias. However, estimates of vaccine effectiveness remained similar in the sensitivity analysis that adjusted for calendar date of RT-PCR sample collection.

In summary, we found that a two-dose schedule of CoronaVac was effective in preventing symptomatic COVID-19 and more severe clinical outcomes among elderly individuals and in a setting with extensive Gamma variant transmission. However, the delayed onset of vaccine-mediated protection underscores the need to prioritise vaccine supplies and maximise the number of individuals who complete the two-dose schedule, when CoronaVac is used as part of a mass vaccination campaign that is implemented in response to a COVID-19 epidemic.

Author contributions

All authors conceived the study. OTR, MDTH and MD completed analyses with guidance from JRA, DATC, AIK, and JC. MSST, OFPP, OTR and MDTH curated and validated the data. OTR and MDTH wrote the first draft of the manuscript. TLD, RCP, OFPP, EFMV, MA, RS, JCG, WNA provided supervision. All authors contributed to, and approved, the final manuscript. JC is the guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Declaration of interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethics approval

The study was approved by the Ethical Committee for Research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021).

Data sharing

Deidentified databases as well as the R codes will be deposited in the repository https://github.com/juliocroda/VebraCOVID-19

Public and Patient Involvement

Members of the public or patients were not involved in setting the research question or the outcome measures, nor were they involved in developing plans for the design of the study. No patients were asked to advise on interpretation or writing up of results.

Transparency statement

The lead author affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained.

Dissemination declaration

Results will be disseminated to the public in Manaus and across Brazil. It is not possible to disseminate results to individuals who were selected into the study due to anonymisation of the data.

Acknowledgements

We are grateful for the Pan American Health Organization's support and the São Paulo State in making the databases available for analysis. JC is supported by the Oswaldo Cruz Foundation (Edital Covid-19 – resposta rápida: 48111668950485). OTR is funded by a Sara Borrell fellowship (CD19/00110) from the Instituto de Salud Carlos III. OTR acknowledges support from the Spanish Ministry of Science and Innovation through the Centro de Excelencia Severo Ochoa 2019-2023 Program and from the Generalitat de Catalunya through the CERCA Program.

Role of the funding source

All funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The Health Secretary of State of São Paulo and PRODESP reviewed the data and findings of the study, but the academic authors retained editorial control. OTR, MDTH, MSST, and JC had full access to de-identified data in the study and OTR and MDTH verified the data, and all authors approved the final version of the manuscript for publication.

References

1 Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *The Lancet Infectious Diseases* 2020; **20**: 533–4.
2 O'Driscoll M, Ribeiro Dos Santos G, Wang L, *et al.* Age-specific mortality and immunity patterns of SARS-CoV-2. *Nature* 2021; **590**: 140–5.
3 Bajaj V, Gadi N, Spihlman AP, Wu SC, Choi CH, Moulton VR. Aging, Immunity, and COVID-19: How Age Influences the Host Immune Response to Coronavirus Infections? *Front Physiol* 2021; **11**: 571416.
4 Collier DA, Ferreira IATM, Kotagiri P, *et al.* Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. *Nature* 2021; published online June 30. DOI:10.1038/s41586-021-03739-1.
5 Palacios R, Batista AP, Albuquerque CSN, *et al.* Efficacy and Safety of a COVID-19 Inactivated Vaccine in Healthcare Professionals in Brazil: The PROFISCOV Study. *SSRN Journal* 2021. DOI:10.2139/ssrn.3822780.
6 Voysey M, Clemens SAC, Madhi SA, *et al.* Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021; **397**: 99–111.
7 Tanriover MD, Doğanay HL, Akova M, *et al.* Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *The Lancet* 2021; : S014067362101429X.
8 Lopez Bernal J, Andrews N, Gower C, *et al.* Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. *BMJ* 2021; : n1088.
9 Dagan N, Barda N, Kepten E, *et al.* BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *N Engl J Med* 2021; : NEJMoa2101765.
10 Strategic Advisory Group of Experts on Immunization - SAGE (WHO). Evidence Assessment: Sinovac/CoronaVac COVID-19 vaccine. Report from 29/04/2021. https://cdn.who.int/media/docs/default-source/immunization/sage/2021/april/5_sage29apr2021_critical-evidence_sinovac.pdf (accessed May 26, 2021).
11 Strategic Advisory Group of Experts on Immunization - SAGE (WHO). Interim recommendations for use of the inactivated COVID-19 vaccine, CoronaVac, developed by Sinovac. 2021; published online May 24. https://apps.who.int/iris/bitstream/handle/10665/341454/WHO-2019-nCoV-vaccines-SAGE-recommendation-Sinovac-CoronaVac-2021.1-eng.pdf.
12 Jara A, Undurraga EA, González C, *et al.* Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *N Engl J Med* 2021; : NEJMoa2107715.
13 Ranzani OT, Bastos LSL, Gelli JGM, *et al.* Characterisation of the first 250 000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. *The Lancet Respiratory Medicine* 2021; **9**: 407–18.
14 Lamarca AP, de Almeida LGP, Francisco R da S, *et al.* Genomic surveillance of SARS-CoV-2 tracks early interstate transmission of P.1 lineage and diversification within P.2 clade in Brazil. Epidemiology, 2021 DOI:10.1101/2021.03.21.21253418.
15 Naveca FG, Nascimento V, de Souza VC, *et al.* COVID-19 in Amazonas, Brazil, was driven by the persistence of endemic lineages and P.1 emergence. *Nat Med* 2021; published online May 25. DOI:10.1038/s41591-021-01378-7.
16 Faria NR, Mellan TA, Whittaker C, *et al.* Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science* 2021; : eabh2644.
17 Jangra S, Ye C, Rathnasinghe R, *et al.* SARS-CoV-2 spike E484K mutation reduces antibody neutralisation. *The Lancet Microbe* 2021; : S2666524721000689.

18 Hoffmann M, Arora P, Groß R, *et al.* SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies. *Cell* 2021; : S0092867421003676.
19 Souza WM, Amorim MR, Sesti-Costa R, *et al.* Neutralisation of SARS-CoV-2 lineage P.1 by antibodies elicited through natural SARS-CoV-2 infection or vaccination with an inactivated SARS-CoV-2 vaccine: an immunological study. *The Lancet Microbe* 2021; : S2666524721001294.
20 GISAID - hCov19 Variants. https://www.gisaid.org/hcov19-variants/ (accessed June 20, 2021).
21 Patel MK, Bergeri I, Bresee JS, *et al.* Evaluation of post-introduction COVID-19 vaccine effectiveness: Summary of interim guidance of the World Health Organization. *Vaccine* 2021; : S0264410X21007076.
22 Freire FHM de A, Gonzaga MR, Gomes MMF. Projeções populacionais por sexo e idade para pequenas áreas no Brasil. *RELAP* 2019; **14**: 124–49.
23 SEADE Foundation - São Paulo State. CORONAVIRUS - CASOS EM SP. Fundação SEADE. https://www.seade.gov.br/coronavirus/ (accessed May 9, 2021).
24 Brazilian Ministry of Health. Campanha Nacional de Vacinação contra a Covid-19. Décimo oitavo informe técnico. https://www.conasems.org.br/wp-content/uploads/2021/04/20a-distribuic%CC%A7a%CC%83o_Campanha_Nacional_de_Vacinacao_contra_a_Covid_19.pdf (accessed May 25, 2021).
25 De Serres G, Skowronski DM, Wu XW, Ambrose CS. The test-negative design: validity, accuracy and precision of vaccine efficacy estimates compared to the gold standard of randomised placebo-controlled clinical trials. *Eurosurveillance* 2013; **18**. DOI:10.2807/1560-7917.ES2013.18.37.20585.
26 Schwartz LM, Halloran ME, Rowhani-Rahbar A, Neuzil KM, Victor JC. Rotavirus vaccine effectiveness in low-income settings: An evaluation of the test-negative design. *Vaccine* 2017; **35**: 184–90.
27 Jackson ML, Chung JR, Jackson LA, *et al.* Influenza Vaccine Effectiveness in the United States during the 2015–2016 Season. *N Engl J Med* 2017; **377**: 534–43.
28 Brazilian Ministry of Health. Epidemiologic Surveillance Guide. National Emergency of Public Health Concern due to the COVID-19 disease. https://www.conasems.org.br/wp-content/uploads/2021/03/Guia-de-vigila%CC%82ncia-epidemiolo%CC%81gica-da-covid_19_15.03_2021.pdf (accessed May 25, 2021).
29 Brazilian Institute of Geography and Statistics - IBGE. Ethinic and race characteristics of the population. Classifications and identities. 2013. https://biblioteca.ibge.gov.br/visualizacao/livros/liv63405.pdf (accessed May 24, 2021).
30 Bueno SM, Abarca K, González PA, *et al.* Interim report: Safety and immunogenicity of an inactivated vaccine against SARS-CoV-2 in healthy chilean adults in a phase 3 clinical trial. Infectious Diseases (except HIV/AIDS), 2021 DOI:10.1101/2021.03.31.21254494.
31 Wu Z, Hu Y, Xu M, *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *The Lancet Infectious Diseases* 2021; : S1473309920309877.
32 Hitchings MDT, Lewnard JA, Dean NE, *et al.* Use of recently vaccinated individuals to detect bias in test-negative case-control studies of COVID-19 vaccine effectiveness. Epidemiology, 2021 DOI:10.1101/2021.06.23.21259415.
33 Sullivan SG, Tchetgen Tchetgen EJ, Cowling BJ. Theoretical Basis of the Test-Negative Study Design for Assessment of Influenza Vaccine Effectiveness. *Am J Epidemiol* 2016; **184**: 345–53.
34 Andrejko KL, Pry J, Myers JF, *et al.* Prevention of COVID-19 by mRNA-based vaccines within the general population of California. Epidemiology, 2021 DOI:10.1101/2021.04.08.21255135.
35 Crowe E, Pandeya N, Brotherton JML, *et al.* Effectiveness of quadrivalent human papillomavirus vaccine for the prevention of cervical abnormalities: case-control study nested within a population based screening programme in Australia. *BMJ* 2014; **348**: g1458–g1458.

36 Moustsen-Helms IR, Emborg H-D, Nielsen J, *et al.* Vaccine effectiveness after 1st and 2nd dose of the BNT162b2 mRNA Covid-19 Vaccine in long-term care facility residents and healthcare workers – a Danish cohort study. Epidemiology, 2021 DOI:10.1101/2021.03.08.21252200.
37 Britton A, Jacobs Slifka KM, Edens C, *et al.* Effectiveness of the Pfizer-BioNTech COVID-19 Vaccine Among Residents of Two Skilled Nursing Facilities Experiencing COVID-19 Outbreaks — Connecticut, December 2020–February 2021. *MMWR Morb Mortal Wkly Rep* 2021; **70**: 396–401.
38 Baum U, Poukka E, Palmu AA, Salo H, Lehtonen TO, Leino T. Effectiveness of vaccination against SARS-CoV-2 infection and Covid-19 hospitalization among Finnish elderly and chronically ill – An interim analysis of a nationwide cohort study. Infectious Diseases (except HIV/AIDS), 2021 DOI:10.1101/2021.06.21.21258686.
39 Yelin I, Katz R, Herzel E, *et al.* Associations of the BNT162b2 COVID-19 vaccine effectiveness with patient age and comorbidities. Infectious Diseases (except HIV/AIDS), 2021 DOI:10.1101/2021.03.16.21253686.
40 Victora C, Castro MC, Gurzenda S, Barros AJD. Estimating the early impact of immunization against COVID-19 on deaths among elderly people in Brazil: analyses of secondary data on vaccine coverage and mortality. Infectious Diseases (except HIV/AIDS), 2021 DOI:10.1101/2021.04.27.21256187.
41 Voysey M, Costa Clemens SA, Madhi SA, *et al.* Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. *Lancet* 2021; **397**: 881–91.
42 Skowronski DM, Setayeshgar S, Zou M, *et al.* Single-dose mRNA vaccine effectiveness against SARS-CoV-2, including P.1 and B.1.1.7 variants: a test-negative design in adults 70 years and older in British Columbia, Canada. Infectious Diseases (except HIV/AIDS), 2021 DOI:10.1101/2021.06.07.21258332.
43 Hitchings MDT, Ranzani OT, Scaramuzzini Torres MS, *et al.* Effectiveness of CoronaVac in the setting of high SARS-CoV-2 P.1 variant transmission in Brazil: A test-negative case-control study. Infectious Diseases (except HIV/AIDS), 2021 DOI:10.1101/2021.04.07.21255081.
44 SoroEpi Group. SoroEpi MSP study in São Paulo Capital - Phase 5 results. https://www.monitoramentocovid19.org (accessed May 17, 2021).

Figure 1. **Incidence of reported COVID-19, vaccination coverage, and prevalence of SARS-CoV-2 variants of concern from Oct 1, 2020 to April 29, 2021 in São Paulo State, Brazil**. Panels A, B, and C show the 14-day rolling average of daily age group-specific incidence of reported COVID-19 cases, hospitalization rate, and mortality (events per 100,000 population), respectively. Panel D shows daily cumulative vaccination coverage in individuals≥70 years of age. Population estimates for age groups were obtained from national projections for 2020.[20] Panel E shows the monthly prevalence of SARS-CoV-2 variants among genotyped isolates in the GISAID database (extraction on June 20th 2021).[18] Vertical bars, from left to right in each panel, show the dates that adults ≥90, 80-89 and 70-79 years of age in the general population became eligible for vaccination.

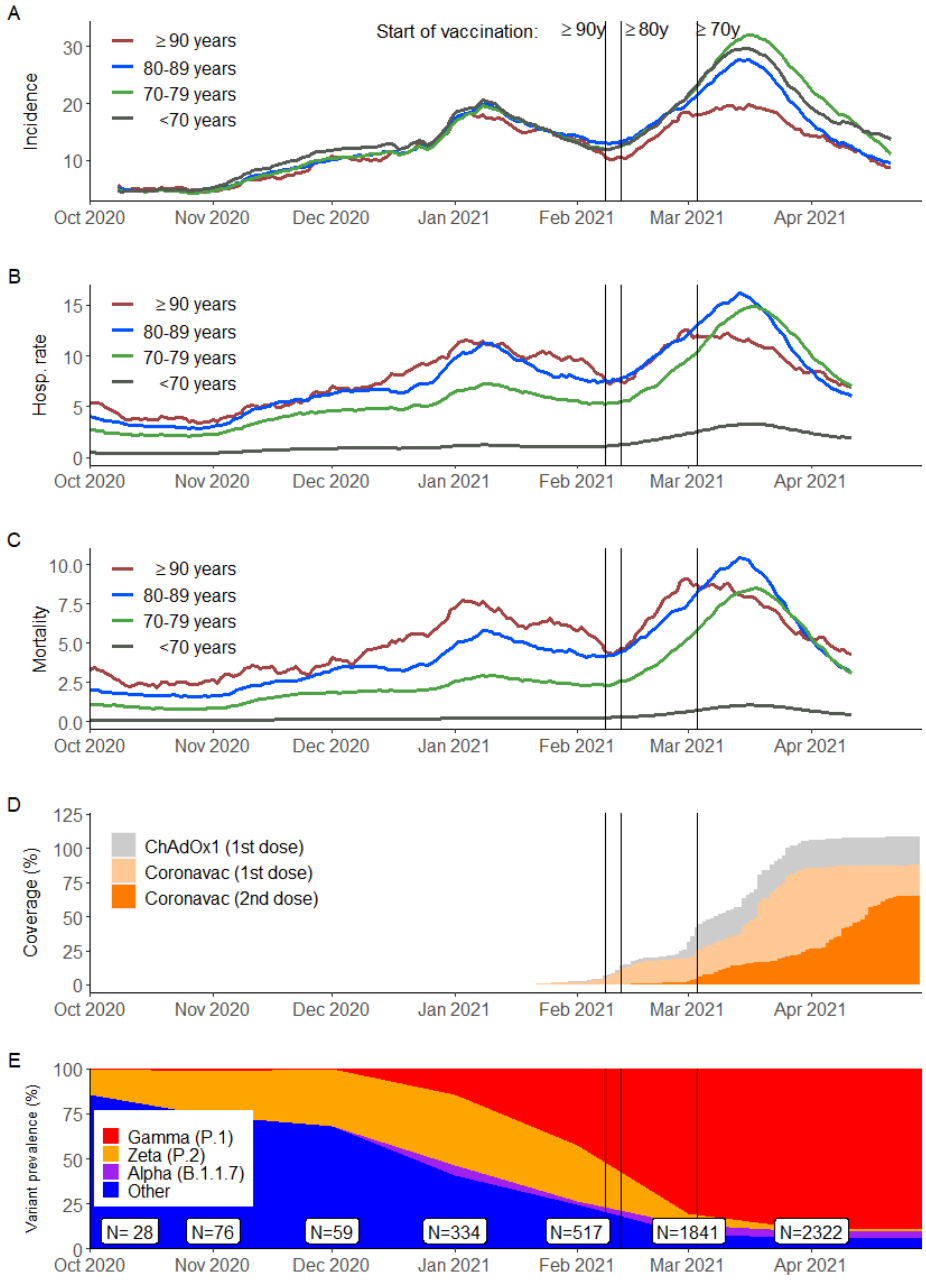


Figure 2. Flowchart of the identification of the study population from surveillance databases and selection of matched cases and controls.

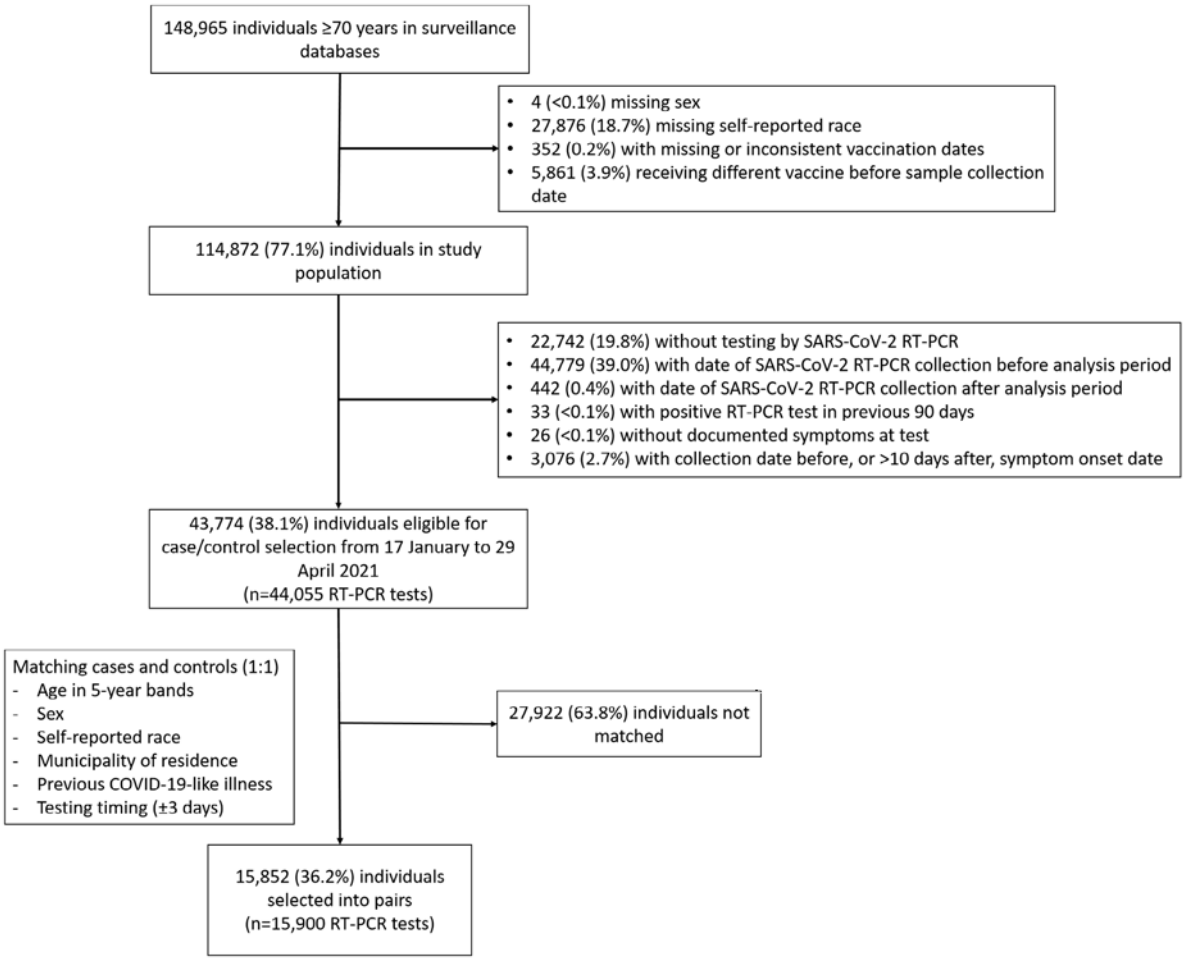


Figure 3. **Adjusted vaccine effectiveness during the period ≥14 days after the second CoronaVac dose for subgroups of adults ≥70 years of age.** Estimates of vaccine effectiveness were obtained from a conditional logistic regression model that included covariates of age and the number of comorbidities and incorporated an interaction term between the category of interest and the period ≥14 days after the second CoronaVac dose.

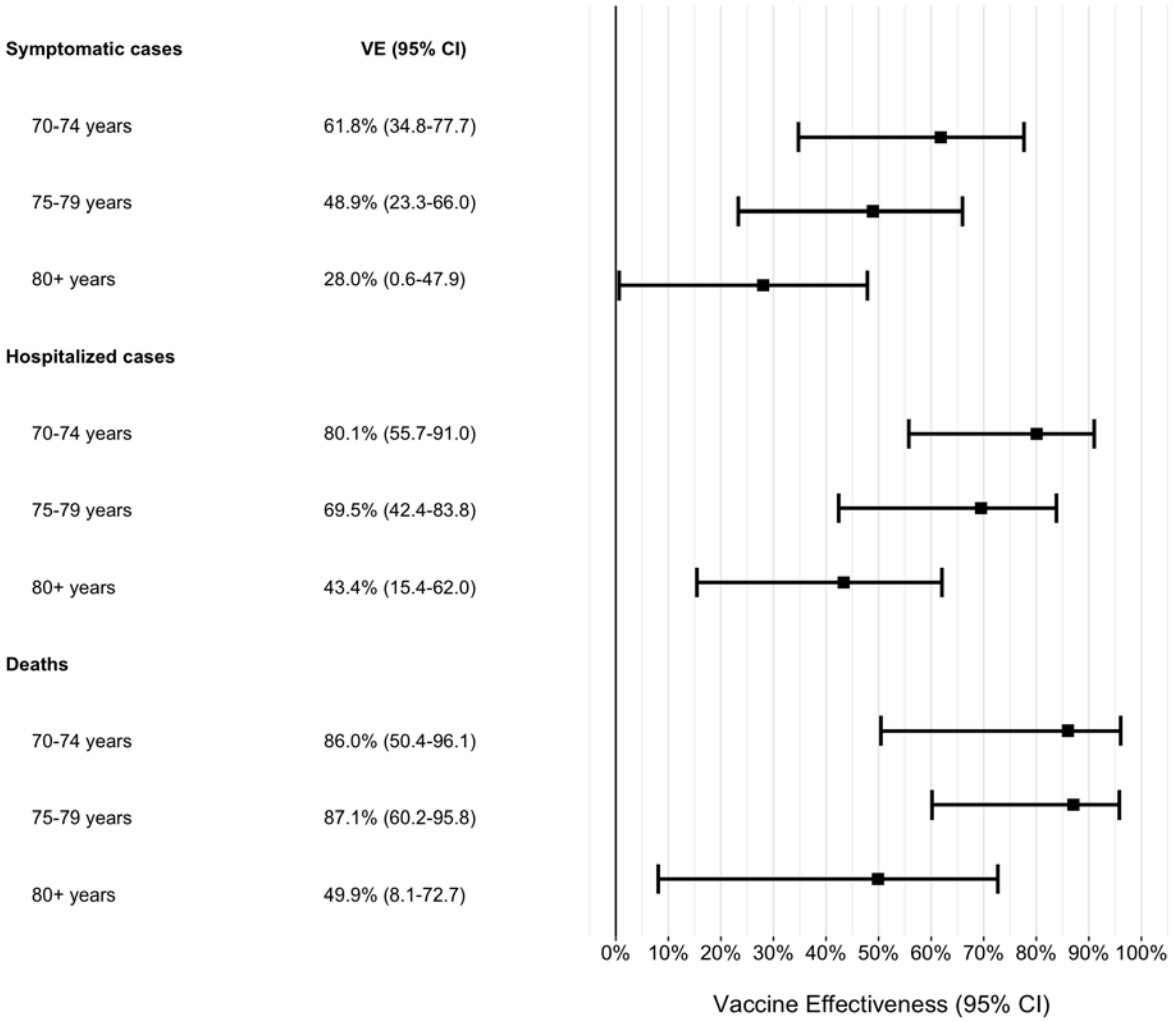


Table 1. Characteristics of adults ≥70 years of age who were eligible for matching and selected into case-test negative pairs.

	Eligible cases and controls		Matched pairs	
Characteristics*	**Test-negative (n=17,622)^**	**Test-positive (n=26,433)^**	**Controls (n=7,950)^**	**Cases (n=7,950)^**
Demographics				
Age, mean (SD), years	77.53 (6.8)	76.71 (6.2)	76.15 (5.8)	76.15 (5.8)
Age categories, n (%)				
70-79 years	12,123 (68.8)	19,673 (74.4)	6,150 (77.4)	6,150 (77.4)
80-89 years	4,301 (24.4)	5,437 (20.6)	1,510 (19.0)	1,510 (19.0)
≥90 years	1,198 (6.8)	1,323 (5.0)	290 (3.6)	290 (3.6)
Male sex, n (%)	7,689 (43.6)	12,431 (47.0)	3,276 (41.2)	3,276 (41.2)
Self-reported race[†], n (%)'				
White/Branca	13,415 (76.1)	19,796 (74.9)	6,420 (80.8)	6,420 (80.8)
Brown/Pardo	3,192 (18.1)	4,983 (18.9)	1,301 (16.4)	1,301 (16.4)
Black/Preta	785 (4.5)	1,258 (4.8)	191 (2.4)	191 (2.4)
Yellow/ Amarela	226 (1.3)	390 (1.5)	38 (0.5)	38 (0.5)
Indigenous/Indigena	4 (0.0)	6 (0.0)	-	-
Residence in "Grande São Paulo" Health Region, n (%)	12,381 (70.3)	16,538 (62.6)	4,259 (53.6)	4,259 (53.6)
Comorbidities				
Reported number[‡], n (%)				
None	10,027 (56.9)	12,668 (47.9)	4,510 (56.7)	3,564 (44.8)
One or two	6,984 (39.6)	12,548 (47.5)	3,151 (39.6)	3,994 (50.2)
Three or more	611 (3.5)	1,217 (4.6)	289 (3.6)	392 (4.9)
Cardiovascular disease , n (%)	5,293 (30.0)	10,079 (38.1)	2,375 (29.9)	3,252 (40.9)
Diabetes, n (%)	3,233 (18.3)	6,533 (24.7)	1,314 (19.0)	2,092 (26.3)
Prior SARS-CoV-2 exposure[**]				

Previous symptomatic events notified to the surveillance systems**, n (%)	685 (3.9)	354 (1.3)	35 (0.4)	35 (0.4)
Positive SARS-CoV-2 test result ††, n (%)	66 (0.4)	13 (0.0)	1 (0.0)	4 (0.1)
Interval between symptoms onset and RT-PCR testing, median (p25-p75), days	3 [2-5]	4 [2-6]	3 [1-5]	4 [2-6]
ARI associated hospitalisations, n (%)	4,524/17,484 (25.9)	12,987/26,221 (49.5)	2,065/7,889 (26.2)	4,039/7,883 (51.2)
ARI associated deaths, n (%)	912/16,710 (5.5%)	7,054/24,508 (28.8%)	729/7,557 (9.6%)	2,052/7,359 (27.9%)
Interval between symptoms onset and hospitalization, median (p25-p75), days	3 [2-6]	7 [4-10]	3 [2-6]	7 [4-10]
Interval between symptoms onset and deaths, median (p25-p75), days	8 [4-13]	14 [9-21]	8 [4-15]	15 [10-22]
Vaccination status				
Not vaccinated, n (%)	11,986 (68.0)	17,233 (65.2)	5,485 (69.0)	5,561 (69.9)
Single dose, within 0-13 days, n (%)	1,446 (8.2)	2,976 (11.3)	747 (9.4)	762 (9.6)
Single dose, ≥14 days, n (%)	1,797 (10.2)	3,312 (12.5)	843 (10.6)	851 (10.7)
Two doses, within 0-13 days, n (%)	1,041 (5.9)	1,533 (5.8)	437 (5.5)	421 (5.3)
Two doses, ≥14 days, n (%)	1,352 (7.7)	1,379 (5.2)	438 (5.5)	355 (4.5)
Interval between first and second dose, mean (SD), days	25 (6)	30 (12)	25 (6)	29 (11)
Interval between first dose and RT-PCR testing, mean (SD), days	28 (19)	23 (16)	24 (17)	23 (16)
Interval between second dose and RT-PCR testing, mean (SD), days	20 (15)	17 (14)	18 (15)	17 (14)

*Continuous variables are displayed as mean (SD); categorical variables are displayed as n (%).
^These numbers refer to RT-PCR tests and represent 43,774 individuals for the eligible cases and controls and 15,852 individuals in the matched cases and controls.
†Race/skin colour as defined by the Brazilian national census bureau (Instituto Nacional de Geografia e Estatísticas).[27]
‡Comorbidities included: cardiovascular, renal, neurological, haematological, or hepatic comorbidities, diabetes, chronic respiratory disorder, obesity, or immunosuppression.
**Prior to the start of the study on 17 January, 2021 and after systematic surveillance was implemented on 1 February, 2020.
** Reported illness with COVID-19 associated symptoms in the eSUS and SIVEP-Gripe databases.
†† Defined as a positive SARS-CoV-2 RT-PCR or antigen detection test result.

Table 2: Effectiveness of CoronaVac against symptomatic COVID-19, hospitalisations and deaths in adults ≥70 years of age.

	Unadjusted Analysis			Adjusted Analysis^		
Symptomatic COVID-19 (n=15,900)	**OR (95% CI)**	**VE (95% CI)**	**p-value**	**OR (95% CI)**	**VE (95% CI)**	**p-value**
Single dose, within 0-13 days vs. unvaccinated*	0.97 (0.85-1.12)	2.7% (-11.7-15.3)	0.70	0.98 (0.85-1.12)	2.5% (-12.2-15.3)	0.72
Single dose, ≥14 days vs. unvaccinated*	0.91 (0.78-1.05)	9.5% (-5.3-22.3)	0.20	0.90 (0.77-1.04)	10.5% (-4.4-23.3)	0.16
Two doses, within 0-13 days vs. unvaccinated*	0.81 (0.66-0.98)	19.5% (1.9-34.0)	0.03	0.82 (0.67-1.00)	18.2% (0.0-33.2)	0.05
Two doses, ≥14 days vs. unvaccinated*	0.60 (0.48-0.74)	40.5% (25.8-52.3)	<0.001	0.58 (0.47-0.73)	41.6% (26.9-53.3)	<0.001
COVID-19 associated hospitalisations (n=8,078)						
Single dose, within 0-13 days vs. unvaccinated*	0.89 (0.74-1.07)	11.3% (-7.0-26.4)	0.21	0.84 (0.68-1.02)	16.4% (-2.2-31.6)	0.08
Single dose, ≥14 days vs. unvaccinated*	0.85 (0.70-1.04)	14.6% (-4.2-30.0)	0.12	0.83 (0.66-1.01)	18.5% (-1.0-34.2)	0.06
Two doses, within 0-13 days vs. unvaccinated*	0.62 (0.47-0.81)	38.1% (18.8-52.8)	0.001	0.59 (0.44-0.79)	40.9% (20.7-55.9)	<0.001
Two doses, ≥14 days vs. unvaccinated*	0.47 (0.36-0.63)	52.7% (37.2-64.4)	<0.001	0.41 (0.30-0.56)	59% (44.2-69.8)	<0.001
COVID-19 associated deaths (n=4,104)						
Single dose, within 0-13 days vs. unvaccinated*	0.92 (0.72-1.18)	8.2% (-17.7-28.4)	0.50	0.93 (0.71-1.21)	7.4% (-21.3-29.2)	0.58
Single dose, ≥14 days vs. unvaccinated*	0.76 (0.57-1.00)	24.5% (0.0-43.0)	0.05	0.68 (0.50-0.93)	31.6% (7.1-49.7)	0.02
Two doses, within 0-13 days vs. unvaccinated*	0.40 (0.27-0.59)	60.4% (40.6-73.5)	<0.001	0.36 (0.23-0.55)	64.4% (44.6-77.1)	<0.001
Two doses, ≥14 days vs. unvaccinated*	0.34 (0.22-0.52)	66.2% (47.8-78.1)	<0.001	0.29 (0.18-0.46)	71.4% (53.7-82.3)	<0.001

ARI - acute respiratory illness

*At date of index sample collection for cases and controls.

^ Models adjusted by age (linear term for symptomatic and hospitalisation, restricted cubic spline for deaths) and number of comorbidities (None, One or Two, Three or more)

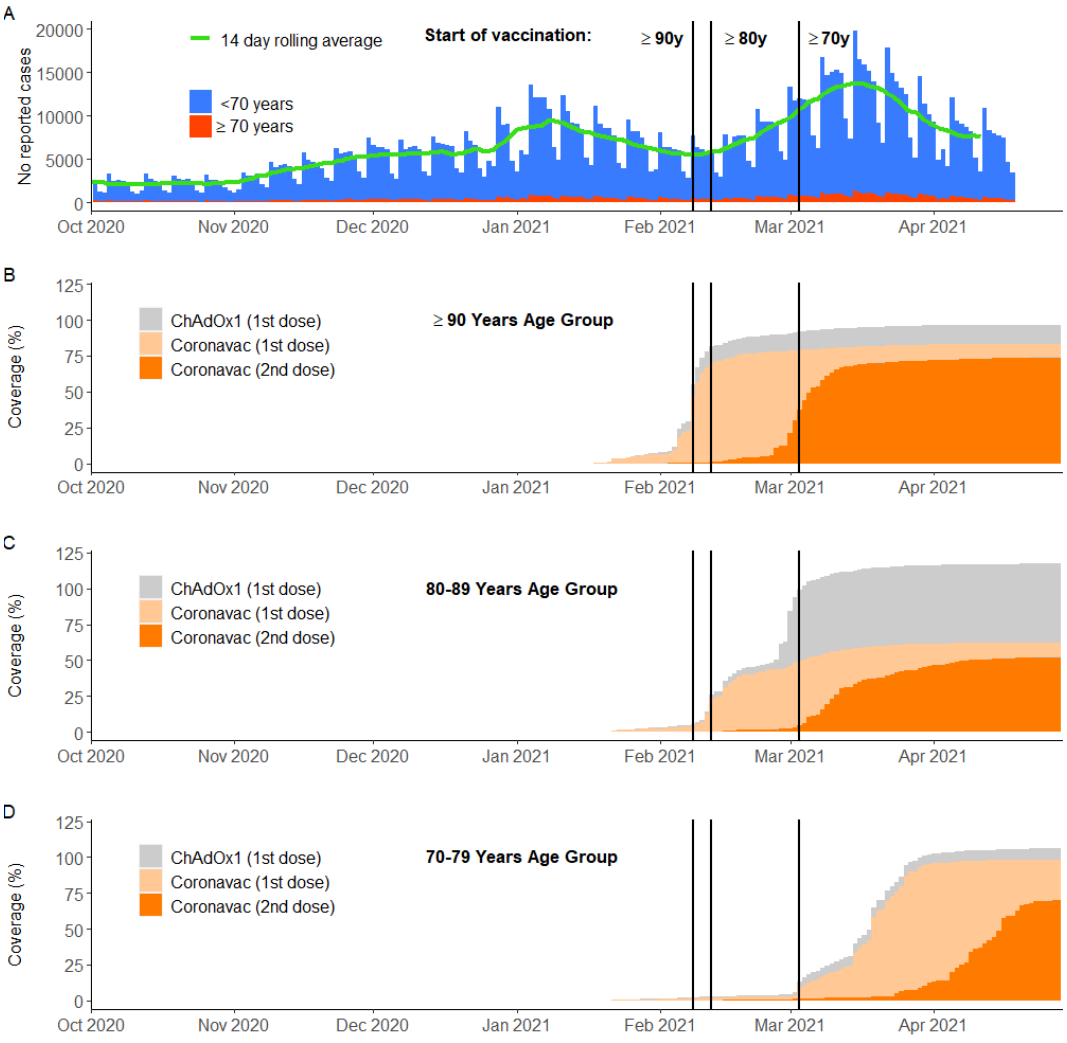
Supplementary appendix

Supplement to: Effectiveness of the CoronaVac vaccine in the elderly population during a Gamma variant-associated epidemic of COVID-19 in Brazil: A test-negative case-control study

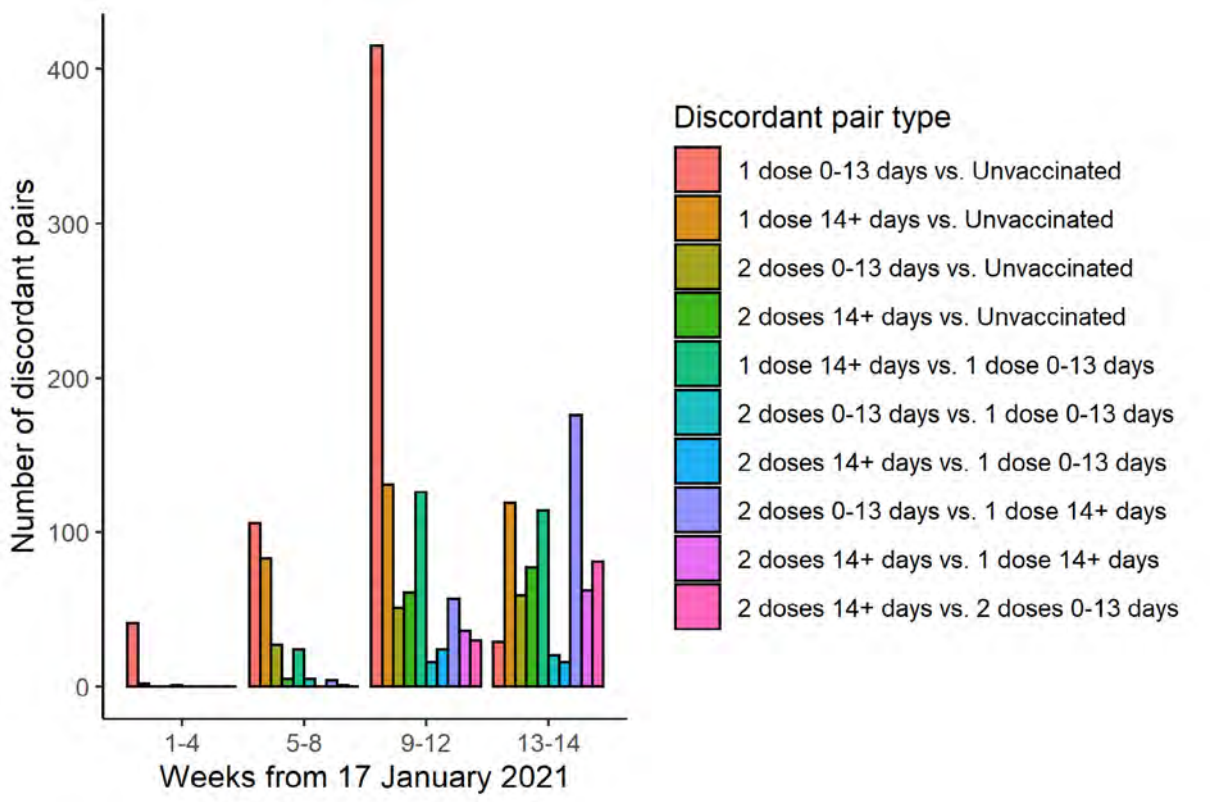
Table of Contents

Supplementary Figure 1. Daily cases and vaccine coverage by age.

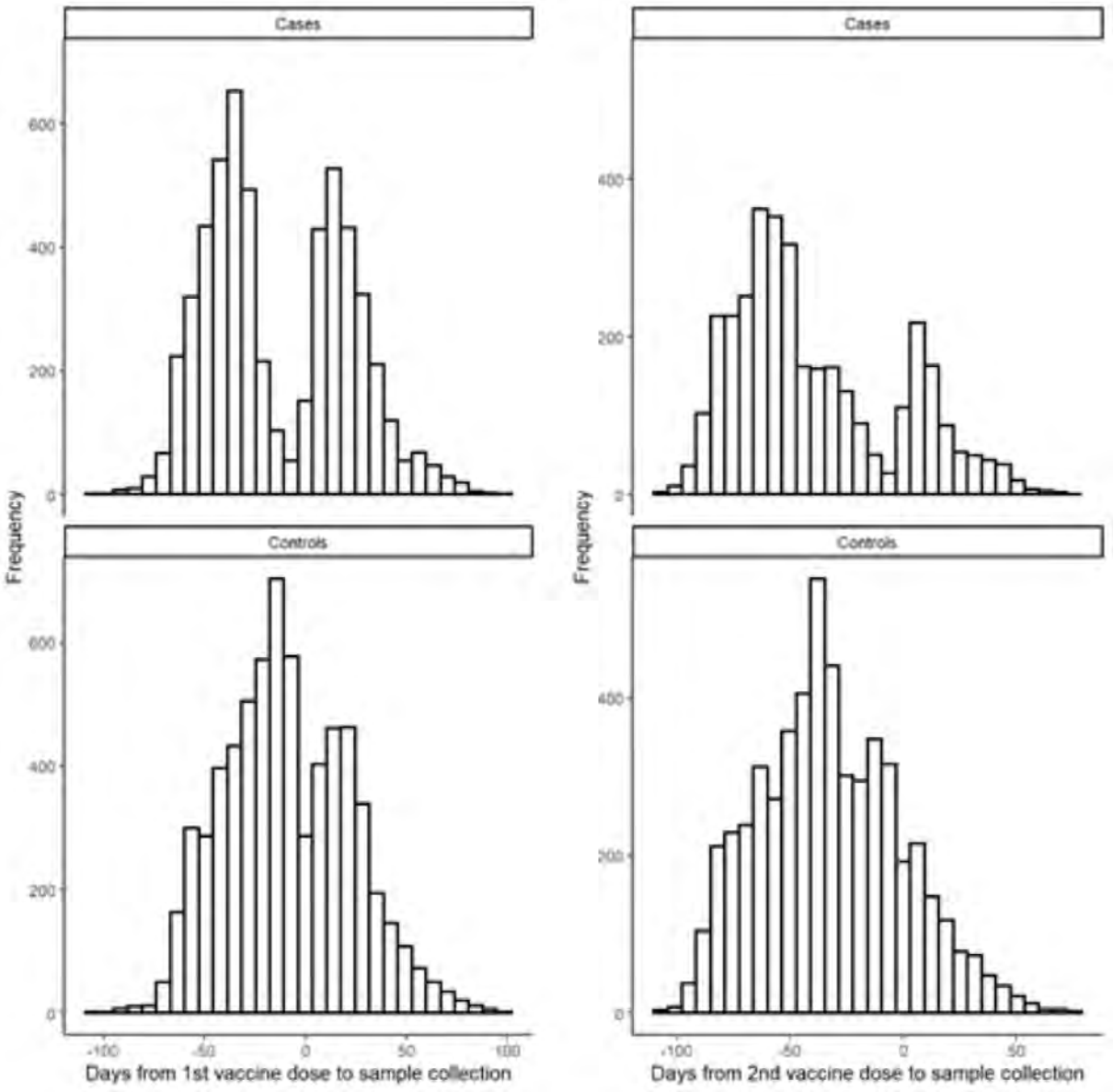
Panel A shows the daily cases of reported COVID-19 from Mar 15, 2020 to Apr 29, 2021 in São Paulo State, Brazil, with the green line representing the 14-day rolling average of counts. Panels B, C and D show the cumulative vaccination coverage for age groups >90y, 80y-89y, and 70y-79y, respectively. Population estimates for age groups were obtained from national projections for 2020.[20] Vertical bars, from left to right in each panel, show the dates that adults ≥90, 80-89 and 70-79 years of age in the general population became eligible for vaccination.



Supplementary Figure 2. Timing of enrolment of discordant case-control pairs by vaccination category



Supplementary Figure 3. Timing of RT-PCR sample collection date relative to first (left column) and second (right column) vaccine dose date, among cases (top row) and controls (bottom row) who were vaccinated during the study period.



Supplementary Table 1. Distribution of concordant and discordant matched case-control pairs.

	Cases				
Controls	Unvaccinated	Single dose, dose 1 within 0-13 days	Single dose, dose 1 ≥14 days	Two doses, dose 2 within 0-13 days	Two doses, dose 2 ≥14 days
Unvaccinated	4,920	290	168	55	52
Single dose, dose 1 within 0-13 days	301	286	131	15	14
Single dose, dose 1 ≥14 days	167	134	379	119	44
Two doses dose 2 within 0-13 days	82	26	118	166	45
Two doses, dose 2 ≥14 days	91	26	55	66	200

Supplementary Table 2. Characteristics of adults ≥70 years of age who were eligible for matching and selected into case-test negative pairs for the hospitalisation analysis.

	Eligible cases and controls		Matched pairs	
Characteristics*	**Test-negative (n=17,622)^**	**Test-positive (n=26,433)^**	**Controls (n=4,039)^**	**Cases (n=4,039)^**
Demographics				
Age, mean (SD), years	77.53 (6.78)	76.71 (6.19)	77.22 (6.41)	77.25 (6.38)
Age categories, n (%)				
70-79 years	12,123 (68.8)	19,673 (74.4)	2847 (70.5)	2847 (70.5)
80-89 years	4,301 (24.4)	5,437 (20.6)	965 (23.9)	965 (23.9)
≥90 years	1,198 (6.8)	1,323 (5.0)	227 (5.6)	227 (5.6)
Male sex, n (%)	7,689 (43.6)	12,431 (47.0)	1771 (43.8)	1771 (43.8)
Self-reported race[†], n (%)'				
White/Branca	13,415 (76.1)	19,796 (74.9)	3251 (80.5)	3251 (80.5)
Brown/Pardo	3,192 (18.1)	4,983 (18.9)	644 (15.9)	644 (15.9)
Black/Preta	785 (4.5)	1,258 (4.8)	115 (2.8)	115 (2.8)
Yellow/ Amarela	226 (1.3)	390 (1.5)	29 (0.7)	29 (0.7)
Indigenous/Indigena	4 (0.0)	6 (0.0)	-	-
Residence in "Grande São Paulo" Health Region, n (%)	12,381 (70.3)	16,538 (62.6)	1783 (44.1)	1783 (44.1)
Comorbidities				
Reported number[‡], n (%)				
None	10,027 (56.9)	12,668 (47.9)	2213 (54.8)	1127 (27.9)
One or two	6,984 (39.6)	12,548 (47.5)	1661 (41.1)	2566 (63.5)
Three or more	611 (3.5)	1,217 (4.6)	165 (4.1)	346 (8.6)
Cardiovascular disease , n (%)	5,293 (30.0)	10,079 (38.1)	1241 (30.7)	2201 (54.5)
Diabetes, n (%)	3,233 (18.3)	6,533 (24.7)	793 (19.6)	1439 (35.6)
Prior SARS-CoV-2 exposure**				

Previous symptomatic events notified to the surveillance systems**, n (%)	685 (3.9)	354 (1.3)	13 (0.3)	13 (0.3)
Positive SARS-CoV-2 test result [††], n (%)	66 (0.4)	13 (0.0)	0 (0.0)	2 (0.0)
Interval between symptoms onset and RT-PCR testing, median (p25-p75), days	3 [2-5]	4 [2-6]	3 [1-5]	4 [2-6]
ARI associated hospitalisations, n (%)	4,524/17,484 (25.9)	12,987/26,221 (49.5)	1,252/4,009 (31.2)	4,039/4,039 (100)
ARI associated deaths, n (%)	912/16,710 (5.5%)	7,054/24,508 (28.8%)	446/3,795 (11.8)	1,939/3,470 (55.9)
Interval between symptoms onset and hospitalization, median (p25-p75), days	3 [2-6]	7 [4-10]	3 [2-6]	7 [4-10]
Interval between symptoms onset and deaths, median (p25-p75), days	8 [4-13]	14 [9-21]	8 [4-15]	15 [10-23]
Vaccination status				
Not vaccinated, n (%)	11,986 (68.0)	17,233 (65.2)	2656 (65.8)	2746 (68.0)
Single dose, within 0-13 days, n (%)	1,446 (8.2)	2,976 (11.3)	413 (10.2)	408 (10.1)
Single dose, ≥14 days, n (%)	1,797 (10.2)	3,312 (12.5)	445 (11.0)	463 (11.5)
Two doses, within 0-13 days, n (%)	1,041 (5.9)	1,533 (5.8)	230 (5.7)	196 (4.9)
Two doses, ≥14 days, n (%)	1,352 (7.7)	1,379 (5.2)	295 (7.3)	226 (5.6)
Interval between first and second dose, mean (SD), days	25 (6)	30 (12)	25 (6)	29 (12)
Interval between first dose and RT-PCR testing, mean (SD), days	28 (19)	23 (16)	25 (19)	24 (18)
Interval between second dose and RT-PCR testing, mean (SD), days	20 (15)	17 (14)	20 (16)	20 (16)

*Continuous variables are displayed as mean (SD); categorical variables are displayed as n (%).

^These numbers refer to RT-PCR tests and represent 43,774 individuals for the eligible cases and controls and 8,059 individuals in the matched cases and controls.

[†]Race/skin colour as defined by the Brazilian national census bureau (Instituto Nacional de Geografia e Estatísticas).

[‡]Comorbidities included: cardiovascular, renal, neurological, haematological, or hepatic comorbidities, diabetes, chronic respiratory disorder, obesity, or immunosuppression.

[**]Prior to the start of the study on 17 January, 2021 and after systematic surveillance was implemented on 1 February, 2020.

** Reported illness with COVID-19 associated symptoms in the eSUS and SIVEP-Gripe databases.

[††] Defined as a positive SARS-CoV-2 RT-PCR or antigen detection test result

Supplementary Table 3. Characteristics of adults ≥70 years of age who were eligible for matching and selected into case-test negative pairs for the death analysis.

	Eligible cases and controls		Matched pairs	
Characteristics*	**Test-negative (n=17,622)^**	**Test-positive (n=26,433)^**	**Controls (n=2,052)^**	**Cases (n=2,052)^**
Demographics				
Age, mean (SD), years	77.53 (6.78)	76.71 (6.19)	77.69 (6.57)	77.76 (6.53)
Age categories, n (%)				
70-79 years	12,123 (68.8)	19,673 (74.4)	1396 (68.0)	1396 (68.0)
80-89 years	4,301 (24.4)	5,437 (20.6)	523 (25.5)	523 (25.5)
≥90 years	1,198 (6.8)	1,323 (5.0)	133 (6.5)	133 (6.5)
Male sex, n (%)	7,689 (43.6)	12,431 (47.0)	962 (46.9)	962 (46.9)
Self-reported race[†], n (%)'				
White/Branca	13,415 (76.1)	19,796 (74.9)	1654 (80.6)	1654 (80.6)
Brown/Pardo	3,192 (18.1)	4,983 (18.9)	320 (15.6)	320 (15.6)
Black/Preta	785 (4.5)	1,258 (4.8)	61 (3.0)	61 (3.0)
Yellow/ Amarela	226 (1.3)	390 (1.5)	17 (0.8)	17 (0.8)
Indigenous/Indigena	4 (0.0)	6 (0.0)	-	-
Residence in "Grande São Paulo" Health Region, n (%)	12,381 (70.3)	16,538 (62.6)	982 (47.9)	982 (47.9)
Comorbidities				
Reported number[‡], n (%)				
None	10,027 (56.9)	12,668 (47.9)	1105 (53.8)	535 (26.1)
One or two	6,984 (39.6)	12,548 (47.5)	868 (42.3)	1304 (63.5)
Three or more	611 (3.5)	1,217 (4.6)	79 (3.8)	213 (10.4)
Cardiovascular disease , n (%)	5,293 (30.0)	10,079 (38.1)	633 (30.8)	1142 (55.7)
Diabetes, n (%)	3,233 (18.3)	6,533 (24.7)	396 (19.3)	754 (36.7)
Prior SARS-CoV-2 exposure[**]				

Previous symptomatic events notified to the surveillance systems**, n (%)	685 (3.9)	354 (1.3)	7 (0.3)	7 (0.3)
Positive SARS-CoV-2 test result [††], n (%)	66 (0.4)	13 (0.0)	0 (0.0)	1 (0.0)
Interval between symptoms onset and RT-PCR testing, median (p25-p75), days	3 [2-5]	4 [2-6]	3 [1-5]	4 [2-6]
ARI associated hospitalisations, n (%)	4,524/17,484 (25.9)	12,987/26,221 (49.5)	645/2,035 (31.7)	1,939/2,025 (95.8)
ARI associated deaths, n (%)	912/16,710 (5.5%)	7,054/24,508 (28.8%)	255/1,940 (13.1)	2,052/2,052 (100)
Interval between symptoms onset and hospitalization, median (p25-p75), days	3 [2-6]	7 [4-10]	3 [2-6]	6 [4-10]
Interval between symptoms onset and deaths, median (p25-p75), days	8 [4-13]	14 [9-21]	8 [4-12]	15 [10-22]
Vaccination status				
Not vaccinated, n (%)	11,986 (68.0)	17,233 (65.2)	1362 (66.4)	1425 (69.4)
Single dose, within 0-13 days, n (%)	1,446 (8.2)	2,976 (11.3)	218 (10.6)	225 (11.0)
Single dose, ≥14 days, n (%)	1,797 (10.2)	3,312 (12.5)	226 (11.0)	236 (11.5)
Two doses, within 0-13 days, n (%)	1,041 (5.9)	1,533 (5.8)	117 (5.7)	79 (3.8)
Two doses, ≥14 days, n (%)	1,352 (7.7)	1,379 (5.2)	129 (6.3)	87 (4.2)
Interval between first and second dose, mean (SD), days	25 (6)	30 (12)	25 (6)	24 (5)
Interval between first dose and RT-PCR testing, mean (SD), days	28 (19)	23 (16)	24 (18)	22 (17)
Interval between second dose and RT-PCR testing, mean (SD), days	20 (15)	17 (14)	19 (16)	20 (15)

*Continuous variables are displayed as mean (SD); categorical variables are displayed as n (%).
^These numbers refer to RT-PCR tests and represent 43,774 individuals for the eligible cases and controls and 4,099 individuals in the matched cases and controls.
[†]Race/skin colour as defined by the Brazilian national census bureau (Instituto Nacional de Geografia e Estatísticas).
[‡]Comorbidities included: cardiovascular, renal, neurological, haematological, or hepatic comorbidities, diabetes, chronic respiratory disorder, obesity, or immunosuppression.
[**]Prior to the start of the study on 17 January, 2021 and after systematic surveillance was implemented on 1 February, 2020.
** Reported illness with COVID-19 associated symptoms in the eSUS and SIVEP-Gripe databases.
[††] Defined as a positive SARS-CoV-2 RT-PCR or antigen detection test result.

Supplementary Table 4. Adjusted vaccine effectiveness during the period ≥14 days after the second CoronaVac dose for subgroups of adults ≥70 years of age.

Estimates of vaccine effectiveness were obtained from a conditional logistic regression model that included covariates of age and the number of comorbidities and incorporated an interaction term between the category of interest and the period ≥14 days after the second CoronaVac dose.

Outcome	OR (95% CI)	VE (95% CI)	p-value for interaction
Symptomatic cases (n=15,900)			
70-74 (n=8,178)	0.38 (0.22-0.65)	61.8% (34.8-77.7)	0.05
75-79 (n=4,122)	0.51 (0.34-0.77)	48.9% (23.3-66.0)	
80+ (n=3,600)	0.72 (0.52-0.99)	28.0% (0.60-47.9)	
Hospitalisations (n=8,078)			
70-74 (n=3,596)	0.20 (0.09-0.44)	80.1% (55.7-91.0)	0.04
75-79 (n=2,098)	0.31 (0.16-0.58)	69.5% (42.4-83.8)	
80+ (n=2,384)	0.57 (0.38-0.85)	43.4% (15.4-62.0)	
Deaths (n=4,104)			
70-74 (n=1,652)	0.14 (0.04-0.50)	86.0% (50.4-96.1)	0.19
75-79 (n=1,140)	0.13 (0.04-0.40)	87.1% (60.2-95.8)	
80+ (n=1,312)	0.50 (0.27-0.92)	49.9% (8.1-72.7)	

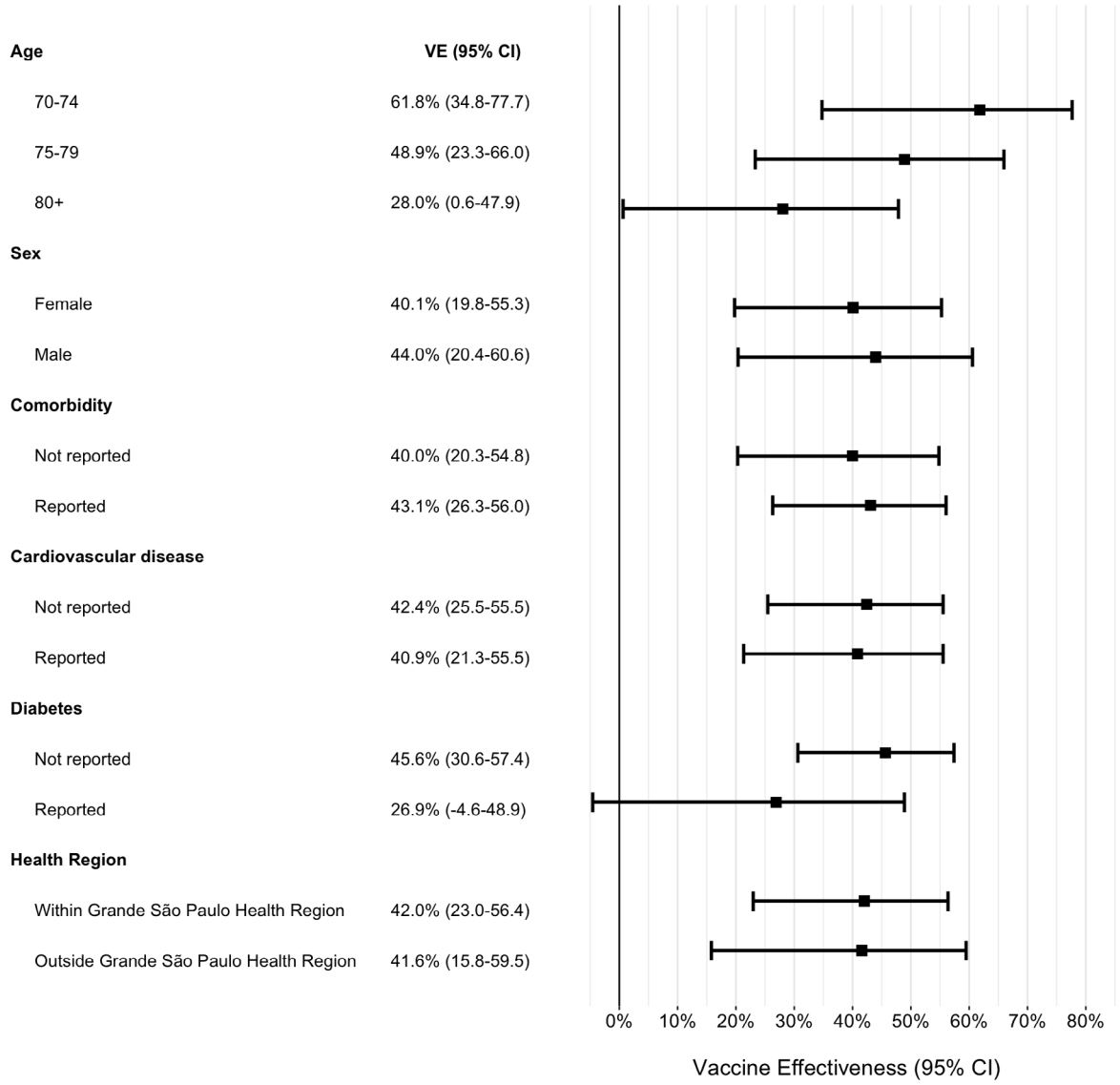
Supplementary Table 5. Estimated effectiveness of CoronaVac ≥14 days after the second dose, in subgroups of adults ≥70 years of age.

All models are adjusted by age (continuous) and number of comorbidities, and include an interaction term between the subgroup of interest and vaccinations with 2 doses, ≥14 days after second vaccine dose.

Subgroup	Adjusted OR (95% CI)	Adjusted VE (95% CI)	p-value for interaction
Age			
70-74 (n=8,178)	0.38 (0.22-0.65)	61.8% (34.8-77.7)	0.05
75-79 (n=4,122)	0.51 (0.34-0.77)	48.9% (23.3-66.0)	
80+ (n=3,600)	0.72 (0.52-0.99)	28.0% (0.60-47.9)	
Sex			
Females (n=9,348)	0.60 (0.45-0.80)	40.1% (19.8-55.3)	0.85
Males (n=6,552)	0.56 (0.39-0.80)	44.0% (20.4-60.6)	
Comorbidities			
No reported (n=8,074)	0.60 (0.45-0.80)	40.0% (20.3-54.8)	0.81
Reported (n=7,826)	0.57 (0.44-0.74)	43.1% (26.3-56.0)	
Cardiovascular disease			
No reported (n=10,273)	0.58 (0.45-0.75)	42.4% (25.5-55.5)	0.86
Reported (n=5,627)	0.59 (0.45-0.79)	40.9% (21.3-55.5)	
Diabetes			
No reported (n=12,294)	0.54 (0.43-0.69)	45.6% (30.6-57.4)	0.12
Reported (n=5,627)	0.73 (0.51-1.05)	26.9% (-4.6-48.9)	
Health regional area			
“Grande São Paulo” (n=7,382)	0.58 (0.44-0.77)	42% (23.0-56.4)	0.66
Not “Grande São Paulo” (n=8,518)	0.58 (0.41-0.84)	41.6% (15.8-59.5)	

Supplementary Figure 4. Adjusted vaccine effectiveness during the period ≥14 days after the second CoronaVac dose for subgroups of adults ≥70 years of age.

Estimates of vaccine effectiveness were obtained from a conditional logistic regression model that included covariates of age (continuous) and the number of comorbidities and incorporated an interaction term between the category of interest and the period ≥14 days after the second CoronaVac dose.



Protocol for the Teste-Negative Case-Control Study in São Paulo State

Version 01.3 / April 30th 2021

Released in https://github.com/juliocroda/VebraCOVID-19/

PROTOCOL

Evaluation of Vaccine Effectiveness in Brazil against COVID-19 (VEBRA-COVID)
Sub-Study: A Test-Negative Case-Control Study on the Effectiveness of COVID-19 Vaccines amongst the General Population of São Paulo State in Brazil

Version: 01.3 / April 30th 2021

Table 1. Protocol Revisions

Changes in Version 1.3	**Justification**
Addition of ChAdOx1 exposure times	We added the time windows following the first and second doses of ChAdOx1 to be 0-13 days, 14-27 days and ≥28 days
Revised expected vaccine effectiveness	In the VEBRA-COVID analysis of the elderly (≥70 years of age) in São Paulo, we aimed to answer the research question of whether vaccines had a real-world effectiveness of public health value rather than whether they had a real-world effectiveness that was consistent with efficacy estimates from RCTs. Thus, we powered the study for a real world effectiveness above a lower threshold of 40%, below which the value of the vaccination would require reconsideration.
Change of matching criteria from CEP (5 digits) to Municipality and self-reported race	We based this decision on three main reasons: 1 – A great proportion of municipalities in São Paulo State has a unique CEP (zipcode), so everyone in that municipality has the same CEP. For these municipalities, we would lose within municipality socioeconomic information 2 – We observed a larger proportion of invalid CEPs mainly in the e-SUS database compared with the SIVEP-Gripe database, which may introduce potential bias since SIVEP-Gripe has a higher proportion of severe COVID-19 cases 3 – A significant number of unique CEPs were inconsistently placed in more than one municipality.
Addition of outcomes for the cohort analysis of test-positive cases	We added ICU admission and respiratory support, occurring within 21 days of initial SARS-CoV-2 test positivity. We also changed hospitalization from occurring within 14 days to within 21 days of initial SARS-CoV-2 test positivity.

I. Background

Since the emergence of severe acute respiratory virus coronavirus 2 (SARS-CoV-2), Brazil has experienced one of the world's highest incidence and mortality rates in the world, with over 13 million reported infections as of the middle of April 2021.[1–3] São Paulo, the most populous state in Brazil (~ 46 million inhabitants), is the state with highest number of cases and deaths: 2,827,833 cases and 92,548 deaths as by April 24th 2021.[4] Variants of Concern (VOC) also had a key role on the recent several surges in Brazil and São Paulo State. The P.1 VOC, which was first detected in Manaus on Jan 12, 2021, [5–7] and now consists the majority of new infections, being dominant in several states in Brazil. P1. has accrued mutations associated with decreased neutralization,[8,9] and has since spread throughout Brazil, synchronizing the epidemic in country in a scenario of relaxed non-pharmacological interventions.

The rapid development of novel vaccines against COVID-19 allowed countries to start vaccine distribution programs within a year of the identification of the novel virus. Among the first vaccines to be developed was Sinovac's CoronaVac vaccine.[10–12] Phase III trials were conducted in Turkey, Chile, Singapore and Brazil. The Brazilian trial was conducted among a study population of healthcare professionals, and reported that the effectiveness of CoronaVac after 14 days following completion of a two dose schedule was 50.7% (95% CI 36.0-62.0) for all symptomatic cases of COVID-19, 83.7% (95% CI 58.0-93.7) for cases requiring medical attention, and 100% (95% CI 56.4-100) for hospitalized, severe, and fatal cases.[12] CoronaVac was approved for emergency use on 17 January in Brazil, and used to vaccinate healthcare workers and the general population. AstraZeneca-Oxford's ChAdOx1 vaccine[13,14] was approved on the same day and was administered beginning on 23 January 2021. In Brazil, ChAdOx1 schedule is for 12 weeks between first and second dose.

As vaccine programs continue, there has been much interest in estimation of vaccine effectiveness through observational studies, and specifically in settings where VOC are circulating. Such studies have advantages over clinical trials, including increased size and follow-up time, and reduced cost. However, as vaccinated and unvaccinated individuals are likely different in their SARS-CoV-2 risk and healthcare access, these studies must address bias through design and analysis. Several studies have demonstrated the effectiveness of COVID-19 vaccines against infection caused by the B.1.1.7 variant.[15] However, large-scale real-world investigations on vaccine effectiveness have not been conducted in regions where the P.1 variant is prevalent.

We propose a test-negative case-control study[16,17] of the general population from the São Paulo State to evaluate the effectiveness of COVID-19 vaccines in preventing symptomatic disease in a setting of widespread P.1 VOC transmission.[6] The study will initially evaluate the effectiveness of COVID-19 vaccines, CoronaVac and ChAdOx1 amongst the population with age ≥70 years, since the vaccination campaign prioritized this age group in its first months. We will expand the study population as additional age groups become eligible for vaccination. Furthermore, we expect that additional vaccines will be approved and will evaluate their effectiveness. We will therefore continue to amend the protocol and its objectives accordingly to address these new questions.

II. Objectives

To estimate the effectiveness of COVID-19 vaccines against symptomatic SARS-CoV-2 infection amongst the general population from the São Paulo State. Our initial analyses will focus on estimating vaccine effectiveness in the age group of ≥70 years.

III. Methods

1. Study Design: We will conduct a retrospective matched case-control study, enrolling cases who test positive for SARS-CoV-2 and controls who test negative for SARS-CoV-2 amongst the general population (Section 3) as of the day that the COVID-19 vaccination campaign was initiated at the study sites. The study will evaluate vaccine effectiveness on the primary outcome of symptomatic SARS-CoV-2 infection. We will identify cases and matched controls by extracting information from health surveillance records and ascertain the type and data of vaccination by reviewing the state COVID-19 vaccination registry. In this design, one minus the odds ratio (1-OR) of vaccination comparing cases and controls estimates the direct effect of vaccination on the disease outcome. In a separate

analysis, we will assess the association between vaccination and hospitalization and/or death among individuals who have tested positive for SARS-CoV-2.

2. IRB and Ethics Statement: The protocol has been submitted to the Ethical Committee for Research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021). The work of investigators at the University of Florida, Yale University, Stanford University, and Barcelona Institute for Global Health was conducted to inform the public health response and was therefore covered under Public Health Response Authorization under the US Common Rule.

Study Details

Study Site: The State of São Paulo (23°3′S, 46°4'W) is the most populous state in Brazil: an estimated population of 46,289,333 in 2020. São Paulo State has 645 municipalities and its capital, São Paulo city, has 12 million inhabitants. São Paulo State reported 2,827,833 COVID-19 cases (cumulative incidence rate: 6,109 per 100,000 population) and 92,548 deaths (cumulative mortality: 200 per 100,000 population), by 24/04/2021. The State Secretary of Health of Sao Paulo (SES-SP) initiated its COVID-19 vaccination campaign on 17 January 2021 and is administering two vaccines, CoronaVac and ChAdOx1. As of 24 April 2021, 10.7 million doses (6.9 million first doses and 3.8 million second doses) have been administered in the State.

Data Sources and Integration: We will identify eligible cases and controls from the State of São Paulo who test positive and negative, respectively, from the *state laboratory testing registry* of public health laboratory network; 2) Determine vaccination status from *state vaccination registries*; and 3) Extract information from *national healthcare and surveillance databases* that will be used to define outcomes, match controls to cases, determine vaccination status, serve as covariates for post-stratification and provide a source for cross-validation of information from databases. Registries are not available which enables constructing a cohort of people eligible for vaccination in the general population. Data sources for this study will include:

- State laboratory testing registry (**GAL**) of the network of public health laboratories
- State COVID-19 vaccination registry (**Vacina Já**)
- National surveillance database of severe acute respiratory illnesses (**SIVEP-Gripe**) created by Ministry of Health Brazil in 2009
- National surveillance system of suspected cases of COVID-19 (**e-SUS**) from mild to moderate "influenza like illness", created by the Ministry of Health Brazil in 2020

The databases will be integrated by the São Paulo State Government – PRODESP - using CPF numbers (Brazilian citizens' unique identifier code) and send to the VEBRA-COVID group anonymized. The database will be updated on a bi-weekly basis.

Study Population

Inclusion criteria:
- Has a residential address in the State of São Paulo,
- Eligible to receive a COVID-19 vaccine based on age,
- With complete information, which is consistent between databases, on age, sex, and residential address
- With consistent vaccination status and dates for those who were vaccinated.

Exclusion criteria:
- Does not have a residential address in the State of São Paulo,
- Not eligible to receive a COVID-19 vaccine based on age,
- With missing or inconsistent information on age, sex, or city of residence
- With existing but inconsistent vaccination status or dates.

Case definition and eligibility: We will use information from integrated GAL/SIVEP-Gripe/e-SUS databases to identify cases that are defined as eligible members of the study population (as defined above, Study Population) who:

- Had a sample with a positive SARS-CoV-2 RT-PCR, which was collected between January 17, 2021 and 7 days prior to database extraction of information
- Did not have a positive RT-PCR test in the 90 day period preceding the index positive RT-PCR result
- Have complete and consistent data on SARS-CoV-2 RT-PCR test results

Control definition and eligibility: We will use integrated GAL/SIVEP-Gripe/e-SUS databases to identify eligible controls. Controls are defined as eligible members of the study population who:

- Had a sample with a negative SARS-CoV-2 RT-PCR result, which was collected after January 17, 2021,
- Did not have a positive RT-PCR test in the 90 day period preceding the index positive RT-PCR result
- Did not have a subsequent positive RT-PCR test in the 7-day period following the index positive RT-PCR result
- Have complete and consistent data on SARS-CoV-2 PCR test result

When studying each vaccine, individuals that received another vaccine are eligible for selection as a case and/or control until the day they receive their vaccine, i.e. we will consider test positive and test negative cases for RT-PCR collected before the day of receipt of the other vaccine.

Matching: Test-negative controls will be matched 1:1 to the cases. We chose the matching factors to balance the ability to reduce bias and to enroll sufficient case-control pairs. Matching factors will include variables that are anticipated to be causes of the likelihood of receiving the vaccine, risk of infection and likelihood of receiving PCR testing for SARS-CoV-2 (see Figures 1-5):

- Age, categorized as 5-years age bands (e.g., 70-74, 75-79 years),
- Sex,
- Municipality,
- Self-reported race,
- Window of ±3 days between collection of RT-PCR positive respiratory sample for cases and collection of RT-PCR negative respiratory sample for controls. If the date of respiratory sample collection is missing, the date of notification of testing result will be used.

We will use the standard algorithms to conduct matching which include: 1) setting a seed, 2) locking the database, 4) creating a unique identifier for matching after random ordering, 5) implementing exact matching based on matching variables, sampling controls at random if more than one available per case within strata.

An individual who fulfils the control definition and eligibility and later has a sample tested that fulfils the case definition and eligibility can be included in the study as both a case and a control. An individual who fulfils the control definition for multiple different sample collection dates can be included in the study as a control for each collection date, up to a maximum of three times.

Exposure definition:
CoronaVac vaccination:

- Received the first vaccine dose, and not having received a second dose, in the following time periods relative to sample collection for their PCR test:
    - 0-13 days
    - ≥14 days
- Received the second dose in the following time periods relative to sample collection for their PCR test:
    - 0-13 days
    - ≥14 days

ChAdOx1vaccination:

- Received the first vaccine dose, and not having received a second dose, in the following time periods relative to sample collection for their PCR test:

  - 0-13 days
  - 14-27 days
  - ≥28 days

- Received the second dose in the following time periods relative to sample collection for their PCR test:
  - 0-13 days
  - ≥14 days

Statistical Analyses: We will evaluate the effectiveness of CoronaVac and ChAdOx1for the following SARS-CoV-2 infection outcomes:

- Primary: Symptomatic COVID-19, defined as one or more reported COVID-19 related symptom with onset within 0-10 days before the date of their positive RT-PCR test

- Secondary:
  - COVID-19 associated hospitalization within 21 days of the symptom onset
  - COVID-19 associated ICU admission within 21 days of the symptom onset
  - COVID-19 associated respiratory support
  - COVID-19 associated death within 28 days of symptom onset

We will evaluate vaccine effectiveness for the primary outcome according to the test-negative design. Table 1 shows a list of all planned analyses in the test-negative design. The test-negative design may introduce bias when evaluating outcomes of hospitalizations and deaths during an epidemic. We will therefore perform time to event/logistic regression analysis of test positive cases to evaluate the association of vaccination status and the risk for hospitalization, ICU admission, COVID-19 respiratory support, and death after infection.

Our initial analyses will focus on estimating vaccine effectiveness in the population with age ≥70 years of age who were the initial priority group of the COVID-19 vaccination campaign.

Case-control analysis: Analyses of the primary outcome will be restricted to case and control pairs who are matched based on the presence of a COVID-19 related symptom before or at the time of testing.

We will use conditional logistic regression to estimate the odds ratio (OR) of vaccination among cases and controls, accounting for the matched design, where 1-OR provides an estimate of vaccine effectiveness under the standard assumptions of a test-negative design. For the CoronaVac analysis, the reference group will be individuals who have not received a first dose of CoronaVac by the date of respiratory sample collection. For the ChAdOx1 analysis, the reference group will be individuals who have not received a first dose of ChAdOx1by the date of respiratory sample collection. Date of notification of the testing result will be used if the date of respiratory sample collection is missing. To evaluate potential biases and the timing of vaccine effectiveness after administration, we will evaluate the windows of vaccination status corresponding: A) 0-13 days and ≥14 days after the 1st dose and 0-13 days and ≥14 days after the 2nd dose of CoronaVac; and B) 0-13 days, 14-27 days and ≥28 after the 1st dose and0-13 days and ≥14 days after the 2nd dose of ChAdOx1.

We will include the following covariates in the adjusted model, which we hypothesize are predictive of vaccination, the risk of SARS-CoV-2 infection and COVID-19 severity and healthcare access and utilization:

- Age as continuous variable
- Comorbidities (None, 1-2, ≥3 comorbidities)
- Evidence of prior SARS-CoV-2 infection (defined as positive PCR test, antigen test or rapid antibody test)

Although data on comorbidities is available through e-SUS and SIVEP-Gripe, this data may have different degrees of missingness between databases and between cases and control groups. Adjusting for comorbidities using complete case data will likely introduce bias. We will explore the feasibility of multiple imputation of comorbidity in a sensitivity analysis. Additional sensitivity analyses will evaluate potential effect modification of the vaccine effectiveness by history of a positive RT-PCR, antigen or serological test result prior to the vaccination campaign and age subgroups.

Survival/logistic regression analysis of hospitalization, ICU, respiratory support and death: We will perform additional analyses for hospitalization and death amongst individuals who test positive and estimate the hazards according to vaccination status at the date of positive test, adjusting for covariates described in the case-control analyses. Sensitivity analyses will be conducted to evaluate the association of influence of a positive RT-PCR, antigen or serological test result prior to the vaccination campaign.

Sample size calculations and timing of analyses: The power of a matched case-control study depends on the assumed odds ratio and the number of discordant pairs (i.e. pairs in which the case is exposed and the control is unexposed, or vice versa), which is a function of the assumed odds ratio and the expected prevalence of exposure among controls. Moreover, the estimate of the odds ratio for one level of a categorical variable compared to baseline is determined by the distribution of all discordant pairs. As vaccine coverage and incidence are changing over time, the latter in ways we cannot predict, and there is no power formula for this analysis, we will simulate power and enroll individuals until we have reached a target power, which we can assess without analyzing the data. In particular, after determining the number of discordant case-control pairs for each combination of exposure categories, we will randomly assign one of each pair to each relevant exposure type according to a Bernoulli distribution, with the probability determined by the assumed odds ratio comparing the two categories. We will run an unadjusted conditional logistic regression on the simulated dataset to determine the p-value, and estimate the power as the proportion of N=1,000 simulations that return $p<0.05$. Code to perform the power calculation can be found at https://github.com/mhitchings/VEBRA_COVID-19.

Timing of final analyses: We will perform an analysis of the primary outcome upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥14 days after the second dose for the CoronaVac. For the ChAdOx1, we will perform an analysis of effectiveness of at least one dose upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥28 days after the first dose. In addition, we will perform an analysis of effectiveness of two doses upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥14 days after the second dose. We chose a vaccine effectiveness of 40% to address the question of whether vaccination with CoronaVac and ChAdOx achieved a threshold of real-world effectiveness, below which the public health value of vaccination may need to be reconsidered.

Privacy: Only SES-SP, São Paulo State data management had access to the identified dataset to linkage the datasets by name, date of birth, mother's name and CPF. After the linkage, the CPF was encrypted and the de-identified dataset was sent to the team for analysis.

Working group: Matt Hitchings, Otavio T. Ranzani, Julio Croda, Albert I. Ko, Derek Adam Cummings, Wildo Navegantes de Araujo, Jason R. Andrews, Roberto Dias de Oliveira, Patricia Vieira da Silva, Mario Sergio Sacaramuzzini Torres, Wade Schulz, Tatiana Lang D Agostini, Edlaine Faria de Moura Villela, Regiane A. Cardoso de Paulo, Olivia Ferreira Pereira de Paula, Jean Carlo Gorinchteyn

References

1 Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *The Lancet Infectious Diseases* 2020; **20**: 533–4.

2 de Souza WM, Buss LF, Candido D da S, *et al.* Epidemiological and clinical characteristics of the COVID-19 epidemic in Brazil. *Nat Hum Behav* 2020; **4**: 856–65.

3 Ranzani OT, Bastos LSL, Gelli JGM, *et al.* Characterisation of the first 250 000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. *The Lancet Respiratory Medicine* 2021; **9**: 407–18.

4 SEADE Foundation - São Paulo State. CORONAVIRUS - CASOS EM SP. Fundação SEADE. https://www.seade.gov.br/coronavirus/ (accessed April 25, 2021).

5 Naveca F, Nascimento V, Souza V, *et al.* COVID-19 epidemic in the Brazilian state of Amazonas was driven by long-term persistence of endemic SARS-CoV-2 lineages and the recent emergence of the new Variant of Concern P.1. In Review, 2021 DOI:10.21203/rs.3.rs-275494/v1.

6 Faria NR, Mellan TA, Whittaker C, *et al.* Genomics and epidemiology of a novel SARS-CoV-2 lineage in Manaus, Brazil. *medRxiv* 2021; published online March 3. DOI:10.1101/2021.02.26.21252554.

7 Fujino T, Nomoto H, Kutsuna S, *et al.* Novel SARS-CoV-2 Variant Identified in Travelers from Brazil to Japan. *Emerg Infect Dis* 2021; **27**. DOI:10.3201/eid2704.210138.

8 Nonaka CKV, Franco MM, Gräf T, *et al.* Genomic Evidence of SARS-CoV-2 Reinfection Involving E484K Spike Mutation, Brazil. *Emerg Infect Dis* 2021; **27**. DOI:10.3201/eid2705.210191.

9 Jangra S, Ye C, Rathnasinghe R, *et al.* The E484K mutation in the SARS-CoV-2 spike protein reduces but does not abolish neutralizing activity of human convalescent and post-vaccination sera. *medRxiv* 2021; published online Jan 29. DOI:10.1101/2021.01.26.21250543.

10 Wu Z, Hu Y, Xu M, *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *The Lancet Infectious Diseases* 2021; : S1473309920309877.

11 Zhang Y, Zeng G, Pan H, *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21**: 181–92.

12 Palacios R, Batista AP, Albuquerque CSN, *et al.* Efficacy and Safety of a COVID-19 Inactivated Vaccine in Healthcare Professionals in Brazil: The PROFISCOV Study. *SSRN Journal* 2021. DOI:10.2139/ssrn.3822780.

13 Folegatti PM, Ewer KJ, Aley PK, *et al.* Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* 2020; **396**: 467–78.

14 Voysey M, Clemens SAC, Madhi SA, *et al.* Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021; **397**: 99–111.

15 Dagan N, Barda N, Kepten E, *et al.* BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *N Engl J Med* 2021; : NEJMoa2101765.

16 Verani JR, Baqui AH, Broome CV, *et al.* Case-control vaccine effectiveness studies: Preparation, design, and enrollment of cases and controls. *Vaccine* 2017; **35**: 3295–302.

17 Verani JR, Baqui AH, Broome CV, *et al.* Case-control vaccine effectiveness studies: Data collection, analysis and reporting results. *Vaccine* 2017; **35**: 3303–8.

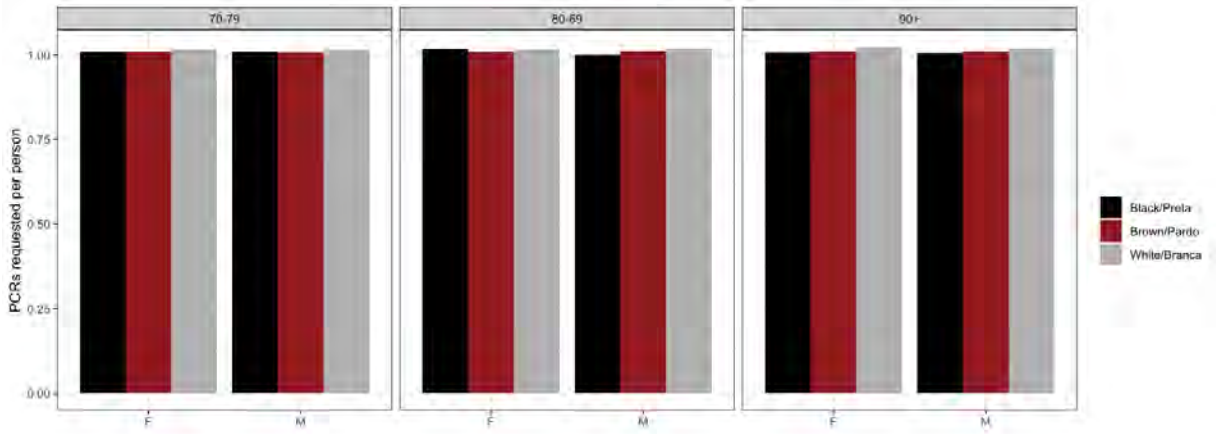


Figure 1: PCR testing rate by age, sex and self-reported race (from data extracted on April 07, 2021)

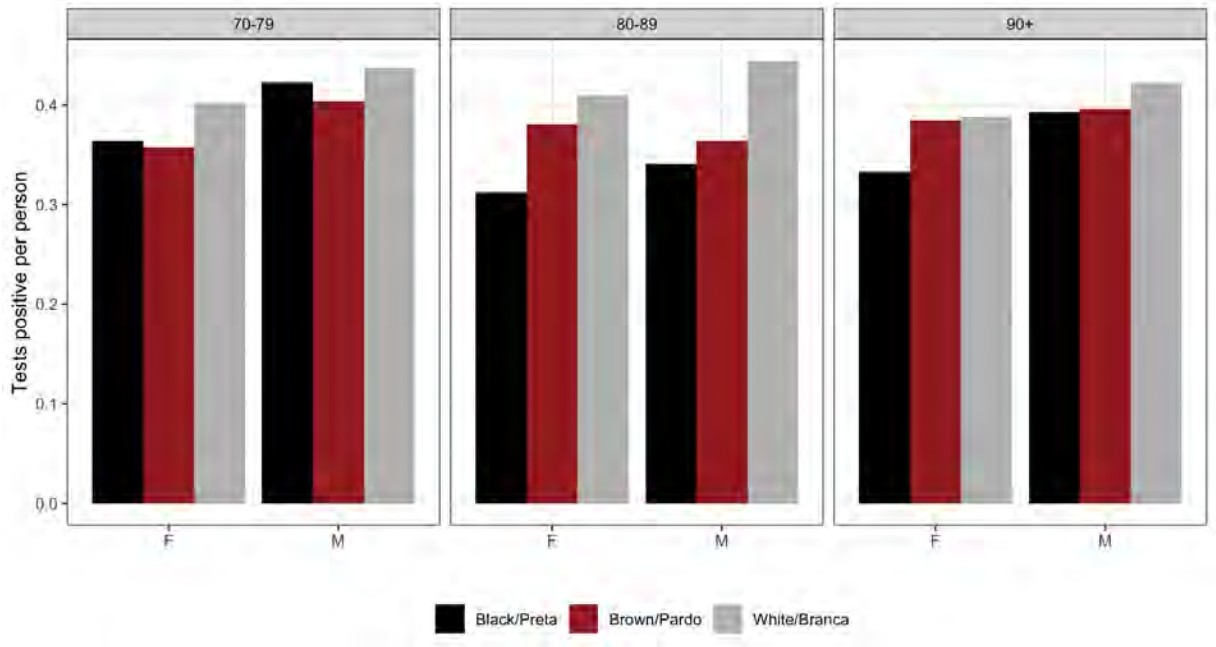


Figure 2: PCR positive testing rate by age, sex and self-reported race (from data extracted on April 07, 2021)

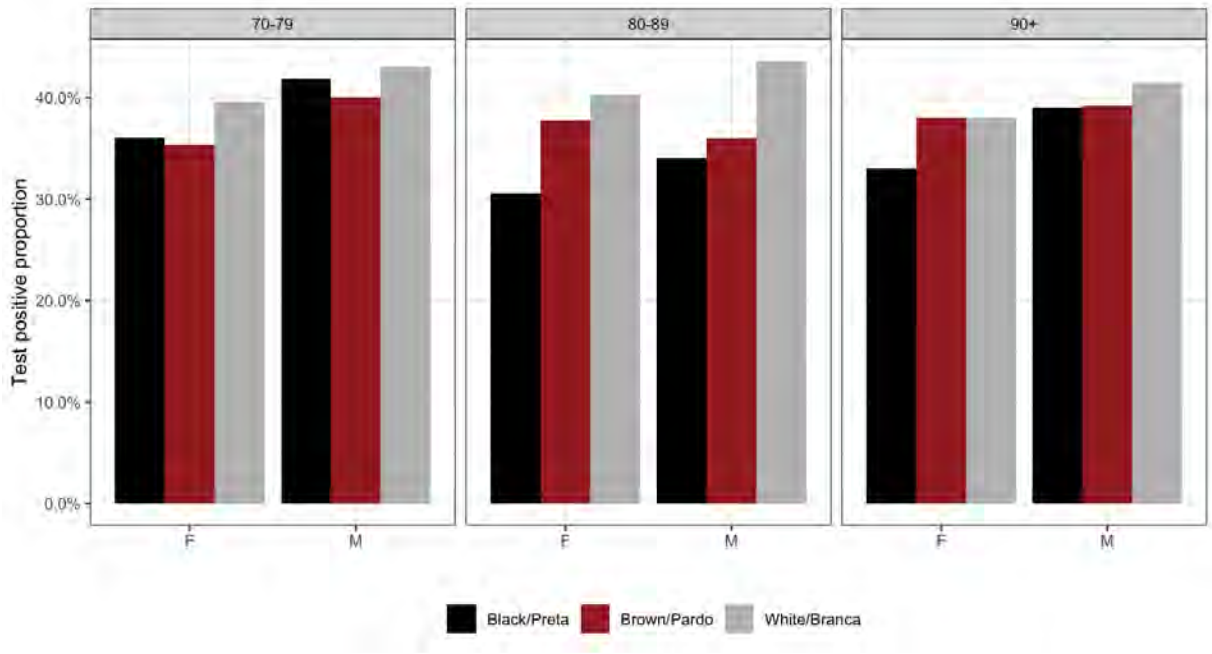


Figure 3: PCR positive proportion by age, sex and self-reported race (from data extracted on April 07, 2021)

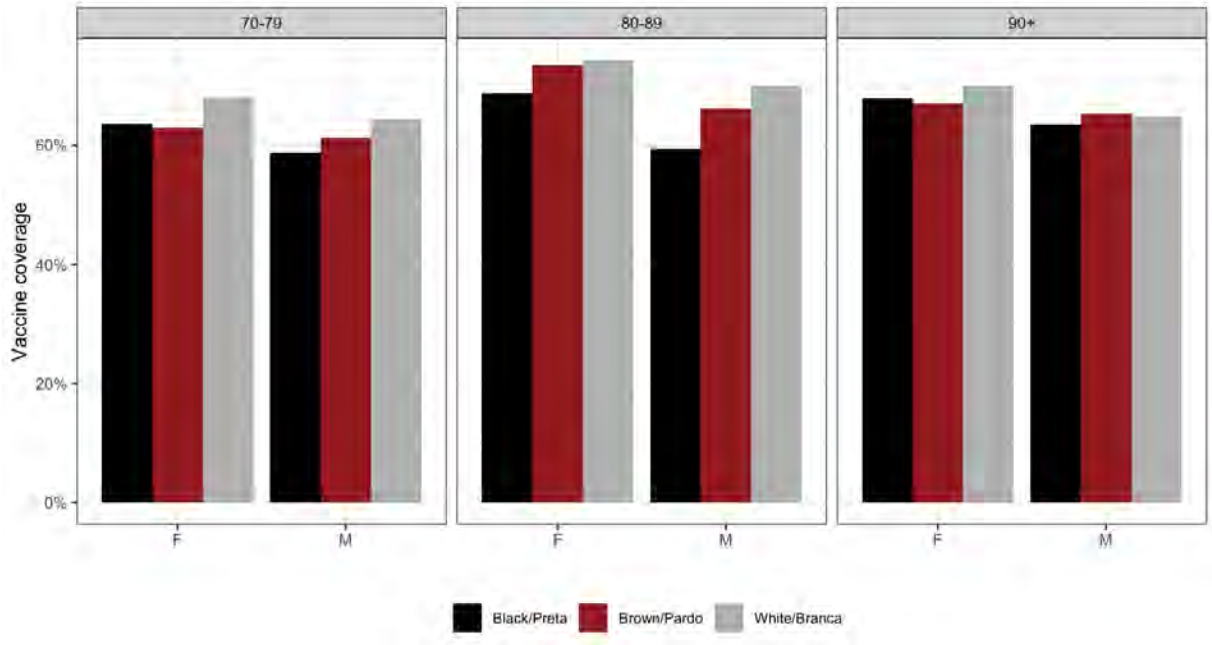
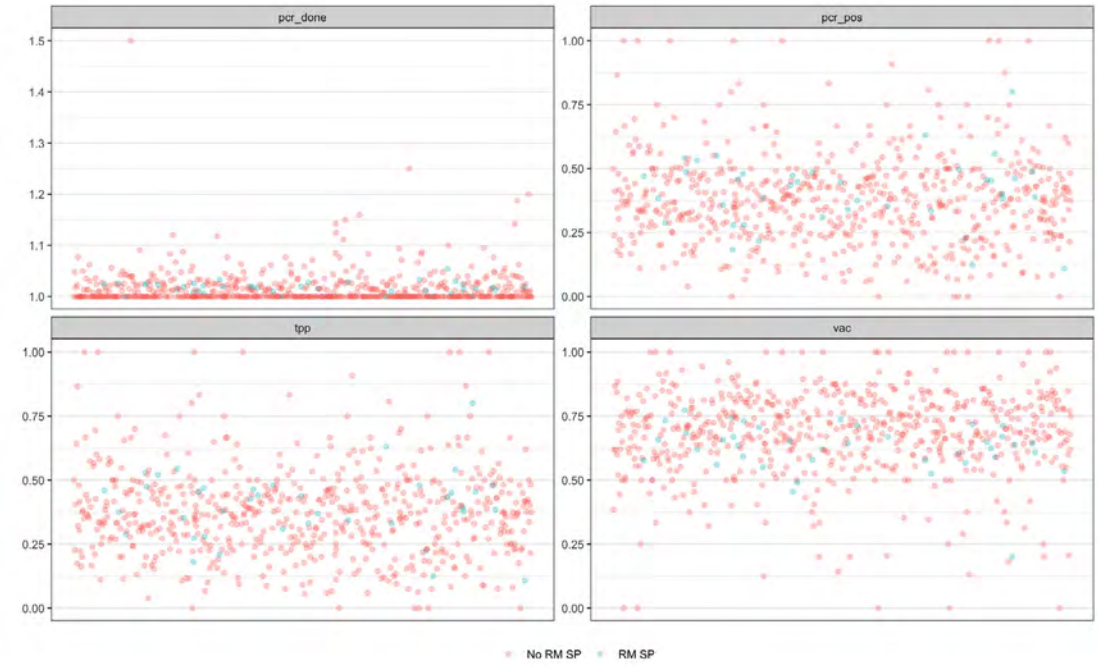


Figure 4: Vaccine coverage by age, sex and self-reported race (from data extracted on April 07, 2021)

Panel A. Indicators by Municipality



Panel B. Indicators by Municipality and Race

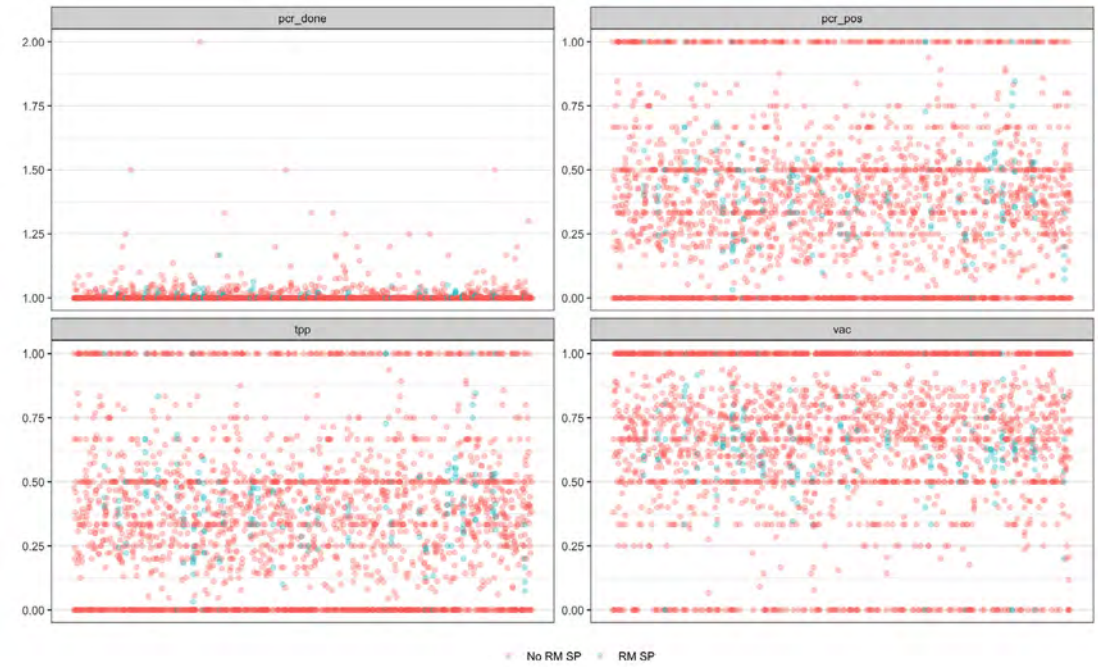
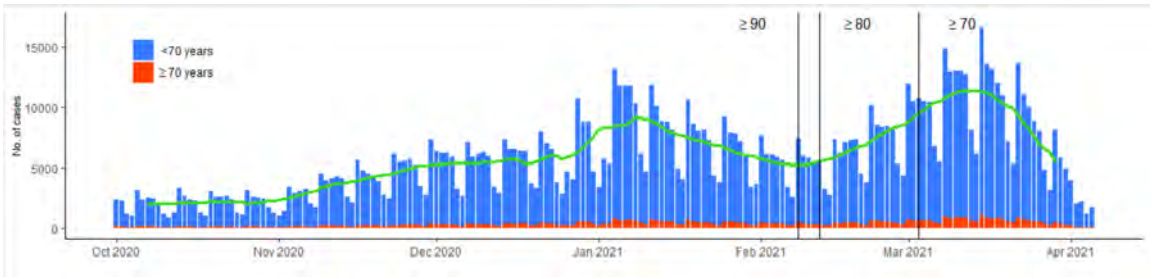


Figure 5: PCR testing rate (pcr_done), PCR positive testing rate (pcr_pos), positivity proportion (tpp) and vaccine coverage (vac) by each municipality (A) and municipality and race (B). RM SP denotes metropolitan area of São Paulo city (from data extracted on April 07, 2021)

Supplementary Figure 1. Reported RT-PCR or Antigen confirmed COVID-19 in the general population of the São Paulo State, Brazil from October 2020 to April 7, 2021. Lines depict moving 14-day averages for case. Vertical lines represent vaccine eligibility by age.



Supplementary Figure 2. Reported RT-PCR or Antigen confirmed COVID-19 rates in the general population of the São Paulo State, Brazil from October 2020 to April 7, 2021. Lines depict rolling averages. Vertical lines represent vaccine eligibility by age.

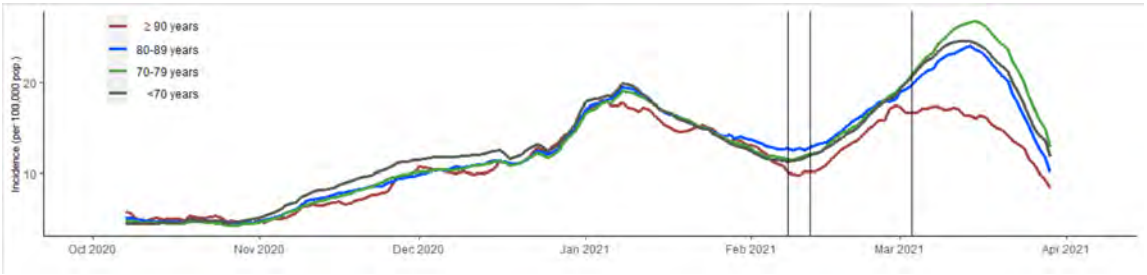


Table 1: Table of planned analyses

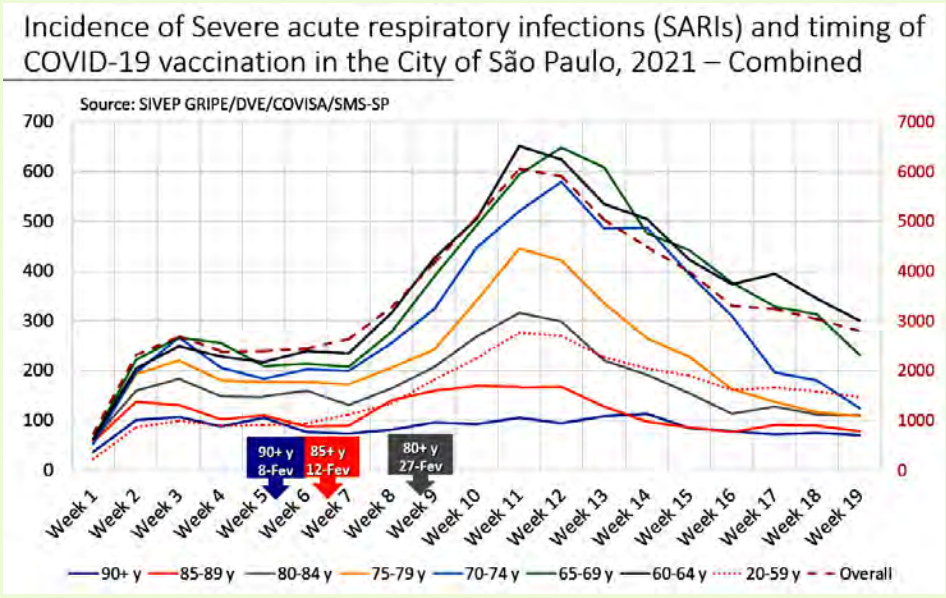
Analysis	Exposure	Outcome
CoronaVac		
Primary outcome, primary exposure	Two-dose regimen of CoronaVac in the period starting 14 days after administration of the 2nd dose	Positive test for SARS-CoV-2, with at least one COVID-19 symptom reported 0-10 days before sample collection date
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of CoronaVac in the period 0-13 days after administration of the 2nd dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of CoronaVac, in the period starting 14 days after administration of the 1st dose	
Primary outcome, bias indicator	One-dose regimen of CoronaVac, in the period 0-13 days after administration of the 1st dose	
ChAdOx1		
Primary outcome, primary exposure	One-dose regimen of ChAdOx1 in the period starting 28 days after administration of the 1st dose	Positive test for SARS-CoV-2, with at least one COVID-19 symptom reported 0-10 days before sample collection date
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of ChAdOx1 in the period ≥14 days after administration of the 2nd dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of ChAdOx1 in the period 0-13 days after administration of the 1st dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of ChAdOx1, in the period starting 14-27 days after administration of the 1st dose	
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of ChAdOx1, in the period starting 0-13 days after administration of the 2nd dose	
Primary outcome, bias indicator	One-dose regimen of ChAdOx1, in the period 0-13 days after administration of the 1st dose	

5.11. CoronaVac está associada à queda da mortalidade de idosos por Covid-19, demonstram estudos

Estudos realizados por pesquisadores do Brasil, dos Estados Unidos e da Espanha demonstraram que a aplicação da CoronaVac, vacina do Butantan contra a Covid-19, levou à queda na internação e nos óbitos por SARS-CoV-2 de pacientes idosos, inclusive em contextos onde predomina a variante gama (P.1) do novo coronavírus.

Segundo o artigo "Estimativa do impacto inicial da imunização contra Covid-19 em mortes entre idosos no Brasil", a escalada da vacinação entre idosos no país está associada a uma queda considerável na mortalidade desse público na comparação com pessoas mais jovens. Na relação entre janeiro-fevereiro (quando poucos idosos haviam tomado a segunda dose) e abril de 2021, a queda no número de mortes na população acima dos 80 anos foi de 25% para 13%.

Entre a primeira semana epidemiológica e o dia 22 de abril de 2021, 171.517 mortes foram atribuídas ao Covid-19 no Sistema de Informações sobre Mortalidade do Ministério da Saúde. O gráfico a seguir mostra que há uma clara aceleração nas mortes e partir da semana 9 (início de março), quando a variante P.1 começa a predominar no Brasil.



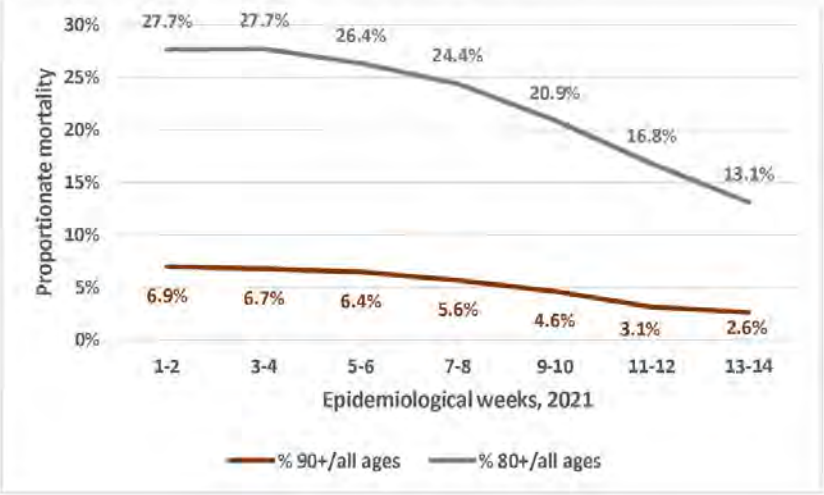
Já entre as semanas epidemiológicas 13 e 14 (em abril, quando cerca de 10 milhões de pessoas haviam recebido a segunda dose), começa a haver uma desaceleração no número de mortes, especialmente em pessoas acima de 70 anos. No gráfico fica evidente que não houve aumento no número de casos positivos no grupo acima de 90 anos, o que demonstra que a vacina se tornou efetiva em conter, neste grupo etário, a força de infecção do vírus.

Além disso, o estudo "Efetividade da vacina CoronaVac na população idosa durante a epidemia de Covid-19 associada à variante P.1 no Brasil", realizado entre janeiro e abril de 2021 com 15 mil casos de pessoas acima dos 70 anos do estado de São Paulo, mostrou que a efetividade da vacina em um contexto onde predomina a variante P.1 aumenta com o tempo e não tem variação significativa em relação à eficácia geral da vacina, sendo de 49,4% 21 dias após a segunda dose. Ela é maior, porém, nos idosos mais jovens: no público entre 70 e 74 anos, a eficácia é de 61,8%.

Dados de efetividade de estudos feitos com o uso da vacina de forma rotineira podem variar e, portanto, devem ser interpretados com cautela. Sem contar que as pesquisas variam do ponto de vista metodológico e analisam momentos epidemiológicos distintos.

É necessário ressaltar que a previsão de eficácia dos estudos está baseada na relação entre os números da

Figure 1. Proportionate mortality of individuals aged 80 or more and 90 or more years relative to deaths at all ages from January to April, Brazil, 2021.



vacinação e os números de casos confirmados e mortes por Covid-19. As pesquisas não se baseiam em indicadores de internação clínica. O objetivo primordial da CoronaVac é reduzir o número de óbitos e internações hospitalares, diminuindo o impacto da doença sobre a perda de vidas e o sistema de saúde.

Estudos realizados no Brasil e em outros países têm demonstrado que a CoronaVac é eficaz contra as novas variantes, comprovadamente a P.1 e a P.2, e que protege todos os grupos etários, inclusive os idosos, contra a mortalidade por Covid-19. Mas é importante salientar que nenhuma vacina impede que uma pessoa seja infectada pelo coronavírus.

Outro ponto relevante é que qualquer vacina gera uma resposta imune menor em pessoas mais idosas. Isso não quer dizer que elas estejam menos protegidas contra a doença, mas sim, que o organismo responde menos a um antígeno novo – uma característica que não se relaciona à efetividade da vacina em si, mas aos processos naturais do sistema imunológico.

Publicado em: 19/06/2021

EClinicalMedicine 38 (2021) 101036

Contents lists available at ScienceDirect

EClinicalMedicine

journal homepage: https://www.journals.elsevier.com/eclinicalmedicine

Research paper

Estimating the early impact of vaccination against COVID-19 on deaths among elderly people in Brazil: Analyses of routinely-collected data on vaccine coverage and mortality

Cesar G. Victora[a,b,*], Marcia C. Castro[b], Susie Gurzenda[b], Arnaldo C. Medeiros[c], Giovanny V.A. França[c], Aluisio J.D. Barros[a]

[a] International Center for Equity in Health, Federal University of Pelotas, Pelotas, RS, Brazil
[b] Department of Global Health and Population, Harvard T.H. Chan School of Public Health, Boston, MA, United States
[c] Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasilia, DF, Brazil

ARTICLE INFO

Article History:
Received 12 May 2021
Revised 29 June 2021
Accepted 1 July 2021
Available online 16 July 2021

Keywords:
COVID-19
Brazil
Immunization
Mortality

ABSTRACT

Background: Vaccination against COVID-19 in Brazil started in January 2021, with health workers and the elderly as the priority groups. We assessed whether there was an impact of vaccinations on the mortality of elderly individuals in a context of wide transmission of the SARS-CoV-2 gamma (P.1) variant.
Methods: By May 15, 2021, 238,414 COVID-19 deaths had been reported to the Brazilian Mortality Information System. Denominators for mortality rates were calculated by correcting population estimates for all-cause deaths reported in 2020. Proportionate mortality at ages 70–79 and 80+ years relative to deaths at all ages were calculated for deaths due to COVID-19 and to other causes, as were COVID-19 mortality rate ratios relative to individuals aged 0–69 years. Vaccine coverage data were obtained from the Ministry of Health. All results were tabulated by epidemiological weeks 1–19, 2021.
Findings: The proportion of all COVID-19 deaths at ages 80+ years was over 25% in weeks 1–6 and declined rapidly to 12.4% in week 19, whereas proportionate COVID-19 mortality for individuals aged 70–79 years started to decline by week 15. Trends in proportionate mortality due to other causes remained stable. Mortality rates were over 13 times higher in the 80+ years age group compared to that of 0–69 year olds up to week 6, and declined to 5.0 times in week 19. Vaccination coverage (first dose) of 90% was reached by week 9 for individuals aged 80+ years and by week 13 for those aged 70–79 years. Coronavac accounted for 65.4% and AstraZeneca for 29.8% of all doses administered in weeks 1–4, compared to 36.5% and 53.3% in weeks 15–19, respectively.
Interpretation: Rapid scaling up of vaccination coverage among elderly Brazilians was associated with important declines in relative mortality compared to younger individuals, in a setting where the gamma variant predominates. Had mortality rates among the elderly remained proportionate to what was observed up to week 6, an estimated additional 43,802 COVID-related deaths would have been expected up to week 19.
Funding: CGV and AJDB are funded by the Todos pela Saúde (São Paulo, Brazil) initiative.

Introduction

In early 2021, Brazil became the global epicenter of the COVID-19 pandemic [1] with an average of over 2000 daily deaths in recent months [2]. The gamma or P.1 variant, initially identified in Manaus in late 2020 [3] has rapidly spread throughout the country [4]. Although genomic analyses are infrequent, in April and May 2021 the new variant accounted for three out of every four samples subjected to viral sequencing [5].

Vaccination against COVID-19 was started in late January 2021, with two types of vaccines being offered: Coronavac (Sinovac, China) and AZD1222 (Oxford-AstraZeneca, UK). Vaccination has been initially targeted at four priority groups: health workers, the elderly (starting with those aged 85 years or more, and gradually vaccinating younger age groups), indigenous populations, and institutionalized individuals. By May 28, 41,478,005 Brazilians had received the first dose, and 19,604,603 the second dose [6].

Vaccination campaigns have been associated with reductions in hospital admissions and mortality among targeted population

* Corresponding author at: International Center for Equity in Health, Federal University of Pelotas, Rua Marechal Deodoro 1160, Pelotas, RS, 96020-220, Brazil
E-mail address: cvictora@equidade.org (C.G. Victora).

https://doi.org/10.1016/j.eclinm.2021.101036

Research in context

Evidence before this study

Brazil has been one of the world's hotspots for COVID-19 in 2021, largely due to the rapid spread of the SARS-CoV-2 gamma variant. Vaccination of the elderly population started in mid-January with the Coronavac (Sinovac, China) and Oxford/AstraZeneca (UK) vaccines. Although the efficacy of both vaccines has been established in phase-3 trials against the original variant of SARS-CoV-2, little is known about their protection against the gamma variant.

Added value of this study

By May 27, 2021, approximately 95% of Brazilians aged 80+ years had received the first vaccine dose. We analyzed data from the Ministry of Health database of over 450,000 COVID-19 deaths since the beginning of the pandemic, including 238,414 deaths in 2021.

Up to mid-February 2021, the deaths of individuals aged 80+ years due to COVID-19 remained almost constant at 25–30% of all reported COVID-19 deaths at any age. Starting in mid-February, proportionate mortality in the elderly started to fall steadily to under 13% in the first half of May. Similar trends were observed for individuals aged 70–79 years, after a time lag that was consistent with the later increase in vaccination coverage in this age group.

Trends in mortality due to other causes were stable, indicating a specific impact on COVID-19 deaths.

Implications of all the available evidence

Confirming early reports from cohorts of vaccinated health workers, our nationwide findings suggest that vaccination against SARS-CoV-2 in Brazil, which largely relied on the Coronavac vaccine in the first trimester of 2021, was associated with an important decline in relative mortality among the elderly compared to younger individuals, in a setting where the gamma variant accounted for three quarters of samples with information on sequencing cases in April-May 2021.

groups, in several of the early starting countries [7-9]. Yet, there is limited evidence on the efficacy of the two vaccines being delivered in Brazil against the gamma variant that currently accounts for the majority of cases in the country. Two observational studies among health care workers in Manaus [10] and São Paulo [11] suggested that the Coronavac provided partial protection against symptomatic illness in settings where gamma accounted for 75% and 47% of all infections, respectively, at the time of the study. Yet, there is growing concern that high SARS-CoV-2 incidence rates such as those observed in Brazil in early 2021 will lead to the appearance of new variants of concern as well as increase in the risk of vaccine escape [12].

To evaluate the real-life effectiveness of the vaccination campaign in Brazil, we analyzed time trends in mortality due to COVID-19 using a database of over 450,000 registered COVID-19 deaths since the beginning of the pandemic. We hypothesized that mortality would fall more rapidly among the elderly, who were the initial target group of the vaccination campaign, than among younger Brazilians.

Methods

Data sources

Data on COVID-19 deaths were obtained from the Ministry of Health Mortality Information System [13] including deaths reported until May 27, 2021. Coverage of the death registration system has been estimated at over 95% by 2010 [14]. As of 2016, the Global Burden of Disease project assigned four out of five stars for the system's coverage and quality of cause of death ascertainment [15], and by 2019 5.6% of all deaths were coded as due to ill-defined causes (França GA, unpublished data). We analyzed deaths for which the underlying cause was coded as B34.2, which included codes U07.1 (COVID-19, virus identified) and U07.2 (COVID-19, virus not identified) [16]. For 84% of 2021 deaths, presence of the virus was confirmed in a laboratory (preliminary results based on investigation of 163,637 deaths).

Data on COVID-19 vaccination coverage were obtained from a dataset made available by the Brazilian Ministry of Health [6]. The data are updated daily and consist of an individual level dataset including personal information and information on the vaccination (type and dose) along with whether it is the first or second dose received and the priority group for the person vaccinated. Data through May 15, 2021 were included in this analysis.

Population estimates

Population estimates for July 1, 2020 by age and sex were obtained from the Brazilian Institute for Geography and Statistics (IBGE) [17]. Due to the excess mortality observed in 2020 and the higher COVID-19 mortality among the elderly [18], the population numbers from IBGE for 2020 are overestimated, particularly at older ages. Since vaccination started in Brazil in early January 2021, it is imperative to obtain an adjusted estimated population that more closely reflects the Brazilian population by the end of 2020. We considered the total deaths that were reported in 2020 (for all causes, as reported in the Mortality Information System), and the expected deaths as implied in the IBGE estimates. We excluded the additional number of deaths from the published 2020 estimates and used that adjusted population as the denominator in our analyses. All adjustments were made by age and sex. All calculations were done in R (R Core Team, 2020).

Data analyses

Mortality results were analyzed in two ways. First, we calculated proportionate mortality by dividing the number of COVID-19 deaths at ages 70–79 and 80+ years by the total number of COVID-19 deaths at all ages. Our main analyses described mortality by epidemiological week in 2021, which are supported by analyses by month of death during 2020. To investigate whether age-specific trends in proportionate mortality were specific to COVID-19 deaths, we also investigated trends due to other causes of death. Second, we calculated COVID-19 age-specific mortality rates by dividing the numbers of weekly deaths from the Mortality Information System by the estimated population by age group, as described above. Mortality rates at ages 70–79 and 80+ years were then divided by rates for the age range 0–9 years in the same week, resulting in mortality rate ratios.

Formal statistical tests were not performed as all results are based on the full country population, rather than samples. Analyses were carried out using Stata version 16 (StataCorp, College Station, TX, USA).

Ethics approval

All analyses were based on anonymized databases that are available at the Brazilian Ministry of Health website [6].

Role of funding source

The funders did not play any role in the preparation of the manuscript, nor on the decision to publish.

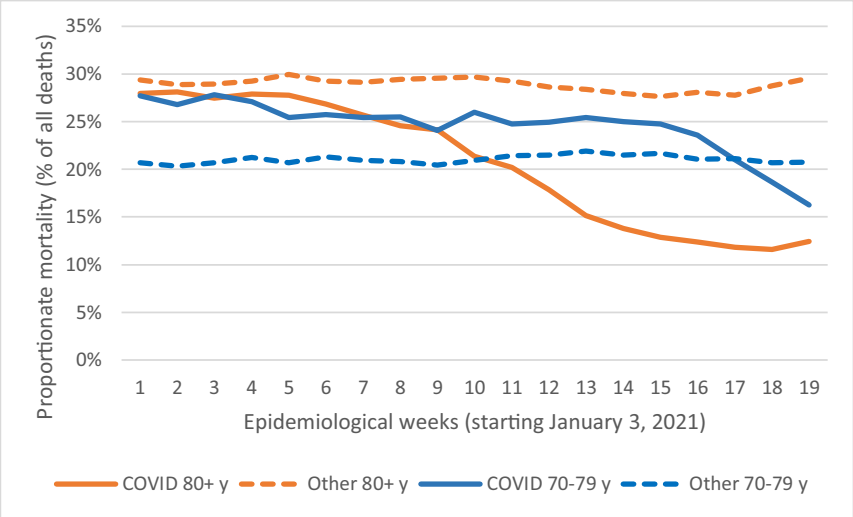


Fig. 1. Proportionate mortality of individuals aged 70–79 and 80 or more years due to COVID-19 and all other causes, relative to deaths due to the same causes at all ages by epidemiological weeks, Brazil, 2021.

Results

From the beginning of the first epidemiological week in 2021 (January 3) to May 15, 238,414 deaths in the Mortality Information System were assigned to COVID-19 and 447,817 to other causes. Supplementary Table 1 shows the absolute number of COVID-19 deaths for epidemiological weeks 1–19 of 2021 (January 3 to May 15). There was rapid acceleration in deaths from week 9 (early March) when the gamma variant became the dominant strain. Results for weeks 17–19 (April 25 to May 15) are likely affected by registration delay but remain useful for comparing age-specific proportionate mortality and mortality rate ratios. Table 1 does not include deaths occurring after epidemiological week 19 (May 16 or later) as these are more markedly affected by delay than earlier deaths.

Fig. 1 shows that proportionate COVID-19 mortality of individuals aged 80+ years fell rapidly from week 6 onwards, whereas proportionate mortality due to non-COVID causes remained relatively stable at just under 30%. Up to May 27, an additional 7,733 deaths had been reported for epidemiological weeks 20 and 21, of which 13.1% were among individuals aged 80+, a finding that is consistent with the levels achieved by week 15. Fig. 1 also shows that proportionate mortality for individuals aged 70–79 years remained at around 25% up to week 15, when it started to decline sharply. For the same age group, proportionate mortality due to other causes remained stable at just over 20% of deaths at any age.

Supplementary Fig. 1 shows that proportionate mortality at ages 80+ years fell in all regions of the country. The trend was less marked in the North region (where the Amazon is located) than in the rest of the country. Supplementary Fig. 2 expands the time series by showing proportionate mortality based on 453,244 COVID-19 deaths that occurred since the beginning of the pandemic in the country. From May 2020 (when the monthly number of deaths exceeded 15,000) to January 2021, proportionate mortality at ages 80+ remained between 25% and 30%, with a sharp reduction starting in mid-February 2021. Proportionate mortality at ages 70–79 years remained above 20% until March 2021, with a substantial decline in April–May. Also showing data for 2020 and 2021, Supplementary Fig. 3 demonstrates that the decline in proportionate mortality was observed for men and women, although proportionate mortality for women aged 80+

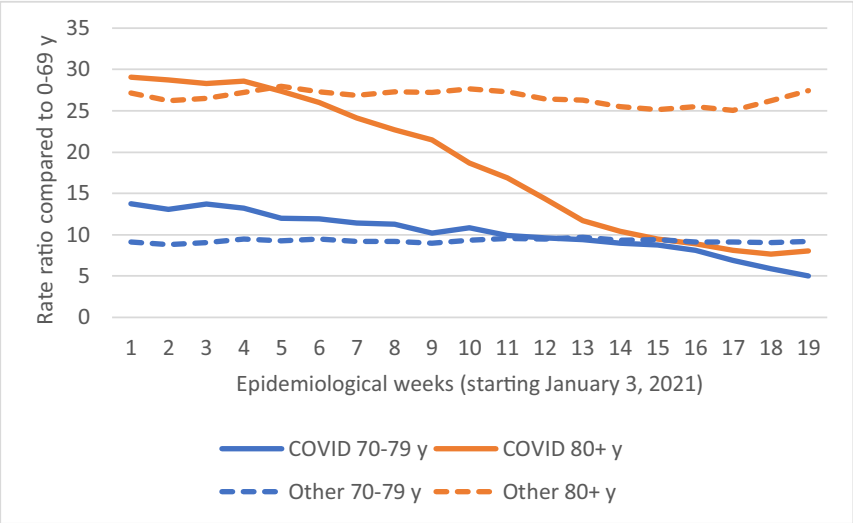


Fig. 2. Mortality rate ratios: mortality rates at ages 70–79 and 80+ years divided by mortality rate at ages 0–69 years by epidemiological weeks, Brazil 2021.

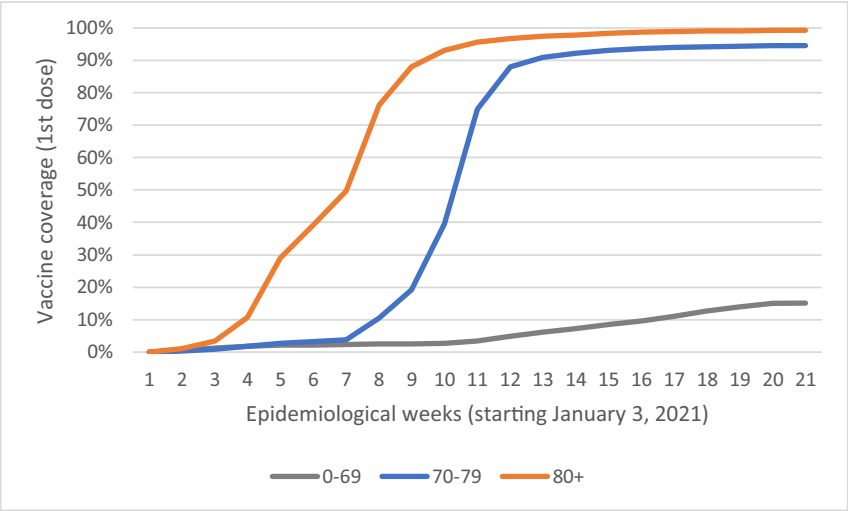


Fig. 3. Covid-19 vaccination coverage (first dose) by age group by epidemiological week, Brazil, 2021.

years tended to be higher than for men, likely due to higher life expectancy of women resulting in fewer deaths in those aged under 80 years.

Fig. 2 shows time trends in mortality rate ratios using the age group 0–69 years as the reference. The mortality rate ratio for persons aged 80+ years fell from over 27 in January and early February to 8 in week 19. The decline in the rate ratio for ages 70–79 was more gradual, from 13.8 in week 1 to 5.0 in week 19. Mortality rate ratios for non-COVID causes remained stable over time.

Fig. 3 shows vaccine coverage for individuals aged 70–79 and 80+ years over time. The increase in coverage was consistent with prioritization of older population groups, with 50% coverage reached for individuals aged 80+ years in the first half of February and over 80% by the second half, stabilizing at around 95% in March. For 70–79-year-olds, 50% coverage was reached by week 11 and 90% coverage by week 19. Coverage among younger age groups was largely restricted to priority groups including health workers, indigenous peoples and people living in institutions. In weeks 1–4, Coronavac accounted for 65.4% of all doses given and AstraZeneca for 29.8% whereas the corresponding percentages for weeks 15–19 were 36.5% and 53.3%. Pfizer/BioNTech (Germany) and Serum Institute (India) accounted for the remaining doses in the recent period.

The downturn in proportionate mortality due to COVID-19 started at about the sixth week of 2021. Had the number of deaths among individuals aged 80+ years continued to increase at the same rate as deaths among people aged 0–69 years, one would expect 70,015 such deaths during the 13-week period from mid-Feb to mid-May. Yet, 32,624 deaths were reported, or 37,401 fewer than expected under the scenario of similar trends as for the 0–69 years age group. A similar calculation was performed for deaths among 70–79-year-olds, among whom proportionate mortality started to decline around week 15. Compared to 13,838 deaths in weeks 15–19, 20,238 would be expected if mortality behaved similarly to that observed for 0–69-year-olds. Adding the two estimates, 43,802 deaths may have been avoided by the decline in mortality among the elderly.

Discussion

We found evidence that, although dissemination of the gamma variant led to increases in reported COVID-19 death at all ages, the proportion of deaths among the elderly started to fall rapidly from the second half of February 2021. This proportion had been stable at around 25–30% since the beginning of the epidemic in early 2020 but is now below 13% in May 2021.

Estimates of proportionate mortality must be interpreted with caution. We now describe how we handled potential caveats in these analyses.

First, the absolute number of deaths in the elderly may be reduced due to smaller number of persons at risk, resulting from high mortality in 2020 due to COVID-19 and other causes. In an estimated population of approximately 815 thousand Brazilians aged 90+ years in 2020, there were approximately 144 thousand deaths in the calendar year, of which about 10% were reported as being caused by COVID-19. To address this potential caveat, our calculations of mortality rates for 2021 were based on population estimates at the beginning of the year from which all-cause deaths had already been deducted.

Second, proportionate mortality may be spuriously reduced among the elderly if the gamma variant of concern disproportionally affected younger individuals, either in terms of infection rates or of infection-fatality rates. The EPICOVID-19 study has been monitoring prevalence of antibodies against SARS-CoV-2 through household surveys in nine large cities in the state of Rio Grande do Sul since April 2020. In early February 2021, antibody prevalence levels were 9.6%, 11.3%, 10.0% and 8.3% for unvaccinated individuals aged 10–19, 20–39, 40–59, and 60+ years, respectively (AJD Barros, personal communication). The state has been strongly affected by the recent pandemic wave, yet there is no evidence of important age patterns in antibody prevalence.

Thirdly, our results based on ratios of mortality rates closely mirror the findings from the proportionate mortality analyses, showing that the rate ratio for individuals aged 80+ relative to those aged 0–69 years fell from 13.3 in January to 8.0 in April.

Lastly, our analyses of deaths due to causes other than COVID-19 showed that proportionate mortality and mortality rate ratios for the elderly remained stable over time, thus supporting the specificity of an impact on COVID-19 deaths.

Another potential limitation of our analyses is the underreporting of deaths and delays in reporting. Delays are particularly relevant for estimating mortality rates for recent periods, as only deaths that reached the system by May 27 were included. However, proportionate mortality by age groups would only be affected if delays varied systematically with age, which is unlikely. As discussed in the Introduction, the overall coverage of mortality statistics has been very high in Brazil for many years, and ill-defined causes represent 5.6% of all deaths. The mortality database for the present analyses includes

approximately 30% more deaths than the SIVEP-Gripe database on hospital admissions and mortality that has been employed in previous analyses of COVID-19 deaths in Brazil [18-20].

However, there is evidence that the excess mortality during 2020 relative to earlier years was not fully explained by deaths due to COVID-19. It is likely that some of such deaths were reported as having been due to other causes or to ill-defined conditions, but it is also possible that increases in non-COVID-19 deaths were because health services were under stress due to the large COVID-19 case load. Unless reporting patterns varied by age or calendar time, this limitation is unlikely to affect the present results particularly in light of the present finding that age patterns in deaths assigned to non-COVID causes remained stable.

The decline in mortality was observed for both sexes. Proportionate mortality at older ages was higher among women than for men, which is compatible with higher case-fatality of younger male adults, possibly related to comorbidities, given that existing serological surveys do not suggest differences in infection prevalence by sex [21,22]. The reductions in proportionate mortality were very similar across four of the five regions of the country. A decline was also observed in the fifth region (Northern Brazil including the Amazon), but proportionate mortality was lower at the beginning of the year than in the rest of the country, and the decline started later than in the rest of the country. The North region has been badly hit by the first and second waves of the pandemic, and high prevalence, high case-fatality, and the limited availability of health services in this region [23] may have led to a larger number of deaths among young adults. Even before the pandemic, life expectancy at birth in the North region was the shortest in the country at 72.9 years, compared to 73.9, 78.3, 78.6 and 75.8 in the Northeast, Southeast, South and Center-West, respectively [17].

The most likely explanation for the observed reductions in proportionate mortality and in rate ratios for the elderly is the rapid increase in vaccination coverage in these age groups, as has been described for other parts of the world [7-9]. The increase in vaccine coverage preceded the decline in mortality, and the decline at ages 80+ years preceded the decline at ages 70–79 years, which is in accordance with the vaccination calendar.

Our results are original in the sense that none of existing population-based mortality studies were carried out in a setting where the gamma variant is predominant. Recent observational studies in vaccinated health workers in Manaus and São Paulo [10,11] had already suggested that Coronavac provided some degree of protection against symptomatic illness in settings where gamma was prevalent. Coronavac accounted for most vaccinations in the 80+ years age group, who were immunized in January and February, with AstraZeneca vaccine accounting for the majority of recent doses. Individuals who received the latter are so far protected by a single dose given that the second dose is provided 12 weeks after the first, whereas the second dose of Coronavac has already been administered to a very high proportion of individuals aged 80+ years [24] as doses are given four weeks apart. The health worker study in São Paulo suggested that the number of cases started to drop after the first Coronavac dose, which is compatible with our findings [11]. This is supported by the results of a recent mass vaccination trial with Coronavac in the town of Serrana (population 27,000) carried out by Instituto Butantan. Following high coverage with Coronavac in early 2021, reductions of 86% in admissions and 95% in deaths were observed in the town by the end of May [25].

We attempted to provide an approximate estimate of lives saved among elderly Brazilians in the eight-week period since vaccination was accelerated throughout the country. The figure of over 40 thousand deaths averted is likely an underestimate, because it does not take into account lives saved among other priority groups for vaccination, such as health workers and indigenous populations. Also, by using the mortality in ages 0–69 years to predict expected deaths among those aged 70+ years, we are not accounting for lives saved by the vaccine among younger age groups – e.g., 60–69-year-olds - for whom coverage also increased, albeit at a slower rate.

Although it is not possible to make strong causal arguments on the basis of the data available for our analyses, our findings are consistent with the results of efficacy trials for both vaccines, with two observational studies in high-risk groups of health workers, [10,11] and with a population-based test-negative study of individuals aged 70+ years in São Paulo State, all of which suggested that vaccination was effective under real-life conditions [26]. Although it is not possible to rule out publication bias, our literature search did not identify any studies showing lack of effectiveness of the Brazilian vaccination campaign, and one would expect that studies showing lack of effectiveness of widely used vaccines would be as likely to be published as those reporting a positive impact. Regarding generalizability, our findings are consistent with the growing evidence of vaccination impact on cases, hospital admissions and deaths in other countries as reported in the lay press [27].

The main contribution brought by the present analyses is to provide large-scale supporting evidence for effectiveness of vaccination in a setting with wide circulation of the gamma variant. Because compliance with non-pharmaceutical interventions such as social distancing and mask use is limited in most of the country, rapid scaling up of vaccination remains as the most promising approach for controlling the pandemic in a country where over 500,000 lives have already been lost to COVID-19 by July 2021.

Funding

CGV and AJDB are funded by the Todos pela Saúde (São Paulo, Brazil) initiative.

Declaration of Interest

The authors declare no competing interest.

Contributors

CGV and MCC conceptualized the manuscript, and CGV wrote the first draft. GVAF and AM extracted the database. AJDB and SG analyzed the data. All authors revised the manuscript and collaborated to produce a revised draft. AJDB and GVAF verified the underlying data. All authors approved the final version.

Data sharing

All data are publicly available on the Brazilian Ministry of Health website [6].

Declaration of Competing Interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2021.101036.

References

[1] Castro MC, Kim S, Barberia L, et al. Spatiotemporal pattern of COVID-19 spread in Brazil. Science 2021.
[2] Brasil. Ministério da Saúde. COVID-19 painel coronavírus. https://covid.saude.gov.br/ (accessed June 16, 2021).
[3] Faria NR, Mellan TA, Whittaker C, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science 2021.

[4] Fundação Oswaldo C. Fiocruz detecta mutação associada a variantes de preocupação no país. March 4, 2021 2021. https://portal.fiocruz.br/noticia/fiocruz-detecta-mutacao-associada-variantes-de-preocupacao-no-pais (accessed June 15, 2021).

[5] GISAID. Genomic epidemiology of hCoV-19. 2021). www.gisaid.org (accessed June 15, 2021).

[6] B M da Saúde. Opendatasus. campanha nacional de vacinação contra COVID-19. https://opendatasus.saude.gov.br/dataset/covid-19-vacinacao (accessed June 15, 2021).

[7] Vasileiou E, Simpson CR, Robertson C, et al. Effectiveness of first dose of COVID-19 vaccines against hospital admissions in Scotland: national prospective cohort study of 5.4 million people, SSRN; 2021.

[8] Israel Ministry of Health. Effectiveness data of the COVID-19 vaccine collected in Israel until 13.2.2021. https://www.gov.il/en/departments/news/20022021-01 (accessed June 15, 2021).

[9] Bernal JL, Andrews N, Gower C, et al. Early effectiveness of COVID-19 vaccination with BNT162b2 mRNA vaccine and ChAdOx1 adenovirus vector vaccine on symptomatic disease, hospitalisations and mortality in older adults in England. medRxiv 2021 03.01.21252652.

[10] Hitchings MDT, Ranzani OT, Scaramuzzini Torres MS, et al. Effectiveness of coronavac in the setting of high SARS-CoV-2 P1 variant transmission in brazil: a test-negative case-control study. MedRxiv 2021 04.07.21255081.

[11] de Faria E, Guedes AR, Oliveira MS, et al. Performance of vaccination with coronavac in a cohort of healthcare workers (HCW) - preliminary report. medRxiv 2021 04.12.21255308.

[12] Thompson RN, Hill EM, Gog JR. SARS-CoV-2 incidence and vaccine escape. Lancet Infect Dis 2021 S1473-3099(21)00202-4.

[13] Brasil. Ministério da Saúde. SIM. sistema de informações de mortalidade. http://www2.datasus.gov.br/DATASUS/index.php?area=060701 (accessed June 15, 2021).

[14] EECd L, Queiroz BL. Evolution of the deaths registry system in Brazil: associations with changes in the mortality profile, under-registration of death counts, and ill-defined causes of death. Cadernos de Saúde Pública 2014;30:1721–30.

[15] Marinho F, de Azeredo Passos VM, Carvalho Malta D, et al. Burden of disease in Brazil, 1990-2013;2016: a systematic subnational analysis for the global burden of disease study 2016. Lancet 2018;392(10149):760–75.

[16] World Health Organization. Emergency use ICD codes for COVID-19 disease outbreak. https://www.who.int/standards/classifications/classification-of-diseases/emergency-use-icd-codes-for-covid-19-disease-outbreak (accessed June 15, 2021).

[17] Instituto Brasileiro de Geografia e Estatística. Projeções da população. https://www.ibge.gov.br/estatisticas/sociais/populacao/9109-projecao-da-populacao.html?=&t=o-que-e (accessed June 15, 2021).

[18] Baqui P, Bica I, Marra V, Ercole A, van der Schaar M. Ethnic and regional variations in hospital mortality from COVID-19 in Brazil: a cross-sectional observational study. Lancet Glob Health 2020;8(8):e1018–e26.

[19] Niquini RP, Lana RM, Pacheco AG, et al. Description and comparison of demographic characteristics and comorbidities in SARI from COVID-19, SARI from influenza, and the Brazilian general population. Cad Saude Publica 2020;36(7):e00149420.

[20] Ranzani OT, Bastos LSL, Gelli JGM, et al. Characterisation of the first 250,000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. Lancet Respir Med 2021;9(4):407–18.

[21] Hallal PC, Hartwig FP, Horta BL, et al. SARS-CoV-2 antibody prevalence in Brazil: results from two successive nationwide serological household surveys. Lancet Glob Health 2020;8(11):e1390–e8.

[22] Hallal PC, et al. Slow spread of SARS-CoV-2 in Southern Brazil over a six-month period: report on eight sequential statewide serological surveys including 35,611 participants. Am J Public Health 2021;29:e1–9.. doi: 10.2105/AJPH.2021.306351. Online ahead of print.

[23] MVd A, ALdÁ V, LDd L, Ferreira MP, Fusaro ER, Iozzi FL. Desigualdades regionais na saúde: mudanças observadas no Brasil de 2000 a 2016. Ciência Saúde Coletiva 2017;22:1055–64.

[24] Brasil. Ministério da Saúde. COVID-19 vacinação - distribuição de vacinas. https://qsprod.saude.gov.br/extensions/DEMAS_C19VAC_Distr/DEMAS_C19VAC_Distr.html (accessed June 15, 2021).

[25] Instituto Butantan. Projeto S: imunização em serrana faz casos de covid-19 despencarem 80% e mortes, 95%. https://butantan.gov.br/noticias/projeto-s-imunizacao-em-serrana-faz-casos-de-covid-19-despencarem-80-e-mortes-95 (accessed June 15, 2021).

[26] Ranzani OT, Hitchings M, Dorion M, et al. Effectiveness of the coronavac vaccine in the elderly population during a P.1 variant-associated epidemic of COVID-19 in Brazil: a test-negative case-control study. medRxiv 2021 05.19.21257472.

[27] Gutiérrez P, A K. Vaccine inequality: how rich countries cut Covid deaths as poorer fall behind. Guardian 2021 June 28, 2021.

CoronaVac

O que a ciência comprova

6. Protege crianças e adolescentes

6.1. Taxa de eventos adversos da CoronaVac em crianças e adolescentes no Brasil é menos de um caso a cada 100 mil doses aplicadas

A taxa de incidência de eventos adversos em crianças e adolescentes que tomaram a CoronaVac no Brasil é de 0,76 para 100 mil doses aplicadas, conforme as notificações recebidas pela Farmacovigilância do Instituto Butantan. Isto é, a notificação de eventos adversos entre os menores de 17 anos que tomaram a vacina do Butantan e da Sinovac foi inferior a um caso a cada 100 mil doses administradas, o que comprova a baixíssima reatogenicidade da CoronaVac na faixa etária de 6 a 17 anos. Os eventos adversos foram relatados espontaneamente para a Farmacovigilância do Instituto Butantan, que fez o levantamento com dados de até o final de julho.

O levantamento leva em conta as mais de 13 milhões de doses da CoronaVac aplicadas nesta faixa etária até o período levantado – 11,05 milhões nas crianças de 6 a 11 anos e 2,02 milhões em adolescentes de 12 a 17 anos.

O Programa Nacional de Imunizações (PNI), do Ministério da Saúde, recomenda o uso da CoronaVac em duas doses, com 28 dias de intervalo entre elas, para crianças, jovens e adultos a partir dos 3 anos de idade. Como a liberação do uso da CoronaVac em menores de 5 anos foi autorizada pela Agência Nacional de Vigilância Sanitária (Anvisa) somente em 13/7, o levantamento conta com dados de vacinação de crianças a partir dos 6 anos, cuja liberação da Anvisa ocorreu em 20/1.

Eventos adversos em crianças de 6 a 11 anos

Segundo o levantamento do Butantan, a ocorrência de eventos adversos em crianças de 6 a 11 anos é ainda mais baixa do que a média geral. O total de 64 casos notificados em crianças desta faixa etária representa apenas 0,58 evento adverso a cada 100 mil doses aplicadas.

Quando se refina este dado, a taxa de incidência de eventos adversos não graves é de 0,38 por 100 mil doses aplicadas. Os eventos com maior incidência são: febre (taxa de incidência de 0,09 por 100 mil doses), vômito (taxa de incidência de 0,04 por 100 mil doses), dor no local da administração (0,03 por

100 mil doses) e cefaleia (0,03 por 100 mil doses).

Já a taxa de incidência de eventos adversos graves é de 0,20 por 100 mil doses aplicadas. Os mais incidentes foram hipersensibilidade e síncope (desmaio), que representam uma taxa de incidência de 0,04 por 100 mil doses.

Eventos adversos em adolescentes de 12 a 17 anos

A ocorrência de eventos adversos em adolescentes de 12 a 17 anos que tomaram as duas doses da CoronaVac também é bastante rara, segundo dados da Farmacovigilância do Instituto coletados no mesmo período. Foram notificados 36 eventos adversos (33 não graves e três graves) entre todos os 2 milhões de adolescentes vacinados, o que equivale a uma taxa de 1,78 evento adverso a cada 100 mil doses aplicadas nesta população.

Entre os eventos adversos não graves notificados, houve dor orofaríngea, hipersensibilidade (ambas com taxas de 0,20 a cada 100 mil doses aplicadas) e tosse pós-vacinação (taxa de 0,15 a cada 100 mil doses aplicadas). Foram ainda notificados casos de mal-estar, febre, espirros (taxa de 0,1 evento adverso a cada 100 mil doses aplicadas), além de dor no local da administração, congestão nasal e prurido no local da administração (taxa de 0,05 evento adverso a cada 100 mil doses aplicadas).

Entre os três eventos adversos graves notificados neste público, e que não foram relacionados à vacinação, um foi de intuscepção (obstrução intestinal), um de enterocolite e um não especificado pelo relator (taxa de 0,05 evento adverso a cada 100 mil doses aplicadas).

"O número total de eventos adversos recebidos espontaneamente pela Farmacovigilância do Instituto Butantan comparados com a estimativa de indivíduos expostos à vacina mostram que a CoronaVac é um produto bastante seguro para estes públicos", afirma a pesquisadora científica e responsável pela Farmacovigilância do Instituto Butantan, Vera Gattás.

6.2. Taxa de eventos adversos da CoronaVac em crianças e adolescentes no Brasil é menos de um caso a cada 100 mil doses aplicadas

Um estudo conduzido pela Fundação Oswaldo Cruz (Fiocruz), publicado na revista Nature Communications, mostrou que a CoronaVac foi capaz de proteger crianças e adolescentes de 6 a 17 anos contra casos graves de Covid-19 durante o surto da variante ômicron. A efetividade estimada foi de 59,2% contra hospitalizações por Covid-19. Os cientistas analisaram dados de quase 200 mil crianças, imunizadas entre janeiro e abril de 2022, após aprovação da vacina do Butantan pela Agência Nacional de Vigilância Sanitária (Anvisa).

Os dados foram obtidos do e-SUS Notifica, sistema nacional de vigilância para RT-PCR e testes de antígeno para infecção por Covid-19, do Sistema de Informação da Vigilância Epidemiológica da Gripe (SIVEP-Gripe) e do Sistema de Informações do Programa Nacional de Imunizações (SI-PNI).

Vale ressaltar que todas as vacinas têm apresentado uma eficiência reduzida contra a infecção pela ômicron, que é mais transmissível, mas os imunizantes têm mantido a sua função principal de prevenir quadros graves e mortes. "Esses achados estão de acordo com estudos anteriores em adultos e adolescentes que mostraram uma redução significativa de efetividade contra ômicron em comparação com as demais variantes", afirmam os autores.

Resultados semelhantes foram observados no Chile, que aplica a vacina no público infantil desde dezembro do ano passado. A efetividade do imunizante foi avaliada em 500 mil crianças de 3 a 5 anos, também durante o período da ômicron. A CoronaVac protegeu 69% contra internação em Unidade de Terapia Intensiva (UTI) e 64,6% contra hospitalização.

Dados de farmacovigilância chilenos também mostraram que a CoronaVac foi o imunizante mais seguro para as crianças e teve a menor taxa de eventos adversos registrada, correspondendo a 0,01% do total de doses administradas. Em conjunto com uma série de estudos, essas evidências serviram de base para a recente ampliação do uso da vacina para a faixa etária de 3 a 5 anos, aprovada pela Anvisa em 13/7. Outros países como China, Colômbia, Tailândia, Camboja, Equador e o território autônomo de Hong Kong já aplicam a CoronaVac nessa população.

A vacinação é a única forma de proteger as crianças contra a Covid-19, que tem causado duas mortes por dia em menores de 5 anos desde o início da pandemia, de acordo com levantamento da Fiocruz.

Publicado em: 13/8/2022

nature communications

Article

https://doi.org/10.1038/s41467-022-32524-5

Vaccine effectiveness of CoronaVac against COVID-19 among children in Brazil during the Omicron period

Received: 28 April 2022

Accepted: 3 August 2022

Published online: 13 August 2022

Check for updates

Pilar T. V. Florentino[1,2] ✉, Flávia J. O. Alves[1], Thiago Cerqueira-Silva[3,4], Vinicius de Araújo Oliveira[1,4], Juracy B. S. Júnior[5], Adelson G. Jantsch[3], Gerson O. Penna[6], Viviane Boaventura[3,4], Guilherme L. Werneck[7,8], Laura C. Rodrigues[9], Neil Pearce[9], Manoel Barral-Netto[1,4], Mauricio L. Barreto[1,10] & Enny S. Paixão[9,10]

Although severe COVID-19 in children is rare, they may develop multisystem inflammatory syndrome, long-COVID and downstream effects of COVID-19, including social isolation and disruption of education. Data on the effectiveness of the CoronaVac vaccine is scarce during the Omicron period. In Brazil, children between 6 to 11 years are eligible to receive the CoronaVac vaccine. We conducted a test-negative design to estimate vaccine effectiveness using 197,958 tests from January 21, 2022, to April 15, 2022, during the Omicron dominant period in Brazil among children aged 6 to 11 years. The estimated vaccine effectiveness for symptomatic infection was 39.8% (95% CI 33.7–45.4) at ≥14 days post-second dose. For hospital admission vaccine effectiveness was 59.2% (95% CI 11.3–84.5) at ≥14 days. Two doses of CoronaVac in children during the Omicron period showed low levels of protection against symptomatic infection, and modest levels against severe illness.

Randomized clinical trials have demonstrated high mRNA vaccine efficacy and immunogenicity in children and adolescents[1,2]. However, data related to the inactivated-virus vaccine (CoronaVac) of efficacy and effectiveness (VE) against the SARS-CoV-2 B.1.1.529 (Omicron) variant are lacking for children aged 6–11 years.

Although severe COVID-19 is a rare condition in children[3], the widespread distribution of SARS-CoV-2 infection and the increasing number of cases in this population has caused a significant public health impact. Besides, children are also susceptible to the multisystem inflammatory syndrome in Children (MIS-C), long-COVID syndrome[3,4] and downstream effects of COVID-19, including social isolation and interruption in education[4]. Therefore, there is an urgent need to collect more data on the effectiveness of vaccines, especially in the Omicron period, to guide decision-makers in adopting policies, such as mandating mask use in school settings.

In Brazil, the children's vaccination campaign started on January 21, 2022[5], and CoronaVac has been used for children aged 6–11 years. On April 15, 2022, vaccine uptake for all vaccines used in children was 62.9% for the 1st dose and 26.6% for the second dose. For CoronaVac, vaccine uptake was 35.1% for 1st dose and 19.8% for the second dose. To our knowledge, no report estimates vaccine effectiveness for CoronaVac among children aged 6–11 years during the Omicron period. Therefore, in this observational study using a nationwide database from Brazil, we estimated the vaccine effectiveness (VE) of the

[1]Centre of Data and Knowledge Integration for Health (CIDACS), Instituto Gonçalo Moniz Institute, Oswaldo Cruz Foundation (Fiocruz), Salvador, Brazil. [2]Biomedical Science Institute, University of São Paulo, São Paulo, Brazil. [3]Gonçalo Moniz Institute, Oswaldo Cruz Foundation (Fiocruz), Salvador, Brazil. [4]Faculty of Medicine, Federal University of Bahia, Salvador, Brazil. [5]Public Health Institute, Federal University of Bahia, Salvador, Brazil. [6]Tropical Medicine Centre, University of Brasília, Fiocruz School of Government, Brasília, Brazil. [7]Department of Epidemiology, Social Medicine Institute, State University of Rio de Janeiro, Rio de Janeiro, Brazil. [8]Institute of Collective Health Studies, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. [9]London School of Hygiene and Tropical Medicine, London, UK. [10]These authors jointly supervised this work: Mauricio L. Barreto, Enny S. Paixão. ✉e-mail: pilar.veras@gmail.com

Article https://doi.org/10.1038/s41467-022-32524-5

CoronaVac against medically attended symptomatic and severe COVID-19 in children aged 6–11 years.

Results

During the study period, 197,958 tests were performed on Brazilian children aged 6–11 years, with 89,595 (45.3%) cases and 108,363 (54.7%) controls, with 508 hospital admissions (Fig. S1). The age, sex, geographic region, socioeconomic position, comorbidities, and hospital admission were similar among the children who tested positive and negative (Table S1). For children between 6 and 11 years, VE against symptomatic COVID-19 during Omicron circulation was 21.2% (95% CI 18.6–23.8) after 13 days post first dose of CoronaVac. After the second dose, VE reached 30.8% (95% CI 24.2–36.8) at 0–13 days and 39.8% (95% CI 33.7–45.4) at ≥14 days (Table 1; Fig. 1) with most of the individuals being tested within 43 days after the second dose (Figure S2). For hospital admission among children vaccinated with one dose of CoronaVac at ≥14 days, the adjusted VE was 47.1% (95% CI 26.6–62.7). After two doses of CoronaVac, the adjusted VE was 82.4% (95% CI 44.2–97.1) at 0–13 days and 59.2% (95% CI 11.3–84.5) at ≥14 days (Table 1; Fig. 1). For ICU admission there were two cases among children vaccinated with two-dose at ≥14 days and the estimated VE for rare events was 20.9% (95% CI [−177.2]–85.0) (Table S2). No death events were detected among children vaccinated with two doses. The sensitivity analyses using multiple imputations for missing data in ethinicity (19.4%) produced similar results to the primary analyses (Table S3). Furthermore, the analyses excluding the previously infected group generated similar VE estimates (Table S4).

Discussion

In this investigation of CoronaVac VE in children 6–11 years of age during Omicron variant predominance, we found that two doses of the CoronaVac vaccine were 39.8% effective against medically attended symptomatic COVID-19 and 59.2% effective in preventing hospital admission COVID-19 cases at ≥14 days after the second dose. The VE estimated in children 6–11 years in Brazil during the Omicron period was much lower than the effectiveness of 75.8% reported for the same demographic in Chile when B.1.617.2 (Delta) was the predominant circulating SARS-CoV-2 variant[6]. However, our data were comparable with results observed in children aged 3–5 during the Omicron outbreak in the same country, 38.2%; (95% CI, 36.5–39.9) against symptomatic disease and, 64.6% (95% CI, 49.6–75.2) against hospitalisation[7]. These findings are also in line with previous studies of VE in adult and adolescent populations that have shown a significant reduction in VE against Omicron compared with early pandemic variants[8,9]. Although we have analysed VE at the optimal period of the second dose among children vaccinated with CoronaVac, it is likely to wane quickly, especially during the Omicron period as it was seen for the adolescent and children population vaccinated with BNT162b2[8,10–13].

This study has strengths and limitations. A strength of this study is the high-quality nationwide database from Brazil. Furthermore, we used Test Negative Design (TND) to minimise bias related to access to health care and health-seeking behaviour. TND's primary assumption is that people seeking and getting tested would be influenced by similar pressures regardless of vaccination status[14]. Another strength is the improbable under ascertainment of vaccination status since the all-vaccines doses administered against COVID-19 in Brazil are recorded in the national immunisation system (SI-PNI). An important limitation is the high rates of asymptomatic infection allied to limited testing in Brazil among children since the database from the study only accounts for tests from the healthcare system and not community testing. Also, the under ascertainment of previous infection may bias the VE estimates if this condition occurs differentially or non-differentially in the vaccinated and unvaccinated group[15–17].

In summary, our findings indicate low levels of protection against symptomatic infection with the Omicron variant after two doses of vaccination with CoronaVac among children. Hence, in line with previous studies involving other vaccines and age groups, the vaccination program alone is unlikely to suppress viral circulation. However, this vaccine was 59.2% effective against COVID-19-hospital admissions, albeit with wide uncertainty intervals. Further studies will be necessary to assess the duration of protection, specially against complications of COVID-19 that occur in the pediatric population, such as MIS-C and long-COVID. Effectiveness also must continue to be monitored as new variants arise.

Methods

Data sources

Data were obtained from three routinely collected sources: the national surveillance system for RT-PCR and antigen tests for COVID-19 infection (e-SUS Notifica); the information system for severe acute respiratory illness (SIVEP-Gripe). These two datasets present notifications from public and private healthcare systems of SARS-CoV-2 suspected cases, and hospitalisation cases of SARS, respectively. Also, the national immunisation system (SI-PNI).

Table 1 | Odds Ratio and Vaccine Effectiveness for Symptomatic Infection and Hospital admission among children aged 6–11 vaccinated with Coronavac

Symptomatic infection					
Vaccination status	Positive tests *n* = 89,595	Negative tests *n* = 108,363	OR Crude (95% CI)	OR adjusted (95% CI)	VE (%) (95% CI)
Unvaccinated	72,737 (50.99%)	69,923 (49.01%)			
1st dose					
0–13 days	7499 (52.22%)	6862 (47.78%)	1.05 (1.02, 1.09)	1.09 (1.05, 1.13)	[−9.0 (−13.1, −4.9)]
≥14-2nd dose	8205 (28.89%)	20,193 (71.11%)	0.39 (0.38, 0.40)	0.79 (0.76, 0.81)	21.2 (18.6, 23.8)
2nd dose					
0–13 days	630 (12.16%)	4552 (87.84%)	0.13 (0.12, 0.14)	0.69 (0.63, 0.76)	30.8 (24.2, 36.8)
≥14 days	524 (7.12%)	6833 (92.88%)	0.07 (0.07, 0.08)	0.60 (0.55, 0.66)	39.8 (33.7, 45.4)
Hospital admission					
Vaccination status	Positive tests *n* = 508	Negative tests *n* = 108,363	OR Crude (95% CI)	OR adjusted (95% CI)	VE (%) (95% CI)
Unvaccinated	428 (0.61%)	69,923 (99.39%)			
1st dose					
0–13 days	30 (0.44%)	6862 (99.56%)	0.71 (0.49, 1.04)	0.73 (0.49, 1.05)	27.0 (−5.2, 51.1)
≥14-2nd dose	42 (0.21%)	20193 (99.79%)	0.34 (0.25, 0.47)	0.53 (0.37, 0.73)	47.1 (26.6, 62.7)
2nd dose					
0–13 days	2 (0.04%)	4552 (99.96%)	0.07 (0.02, 0.29)	0.18 (0.03, 0.56)	82.4 (44.2, 97.1)
≥14 days	6 (0.09%)	6833 (99.91%)	0.14 (0.06, 0.32)	0.41 (0.16, 0.89)	59.2 (11.3, 84.5)

 https://doi.org/10.1038/s41467-022-32524-5

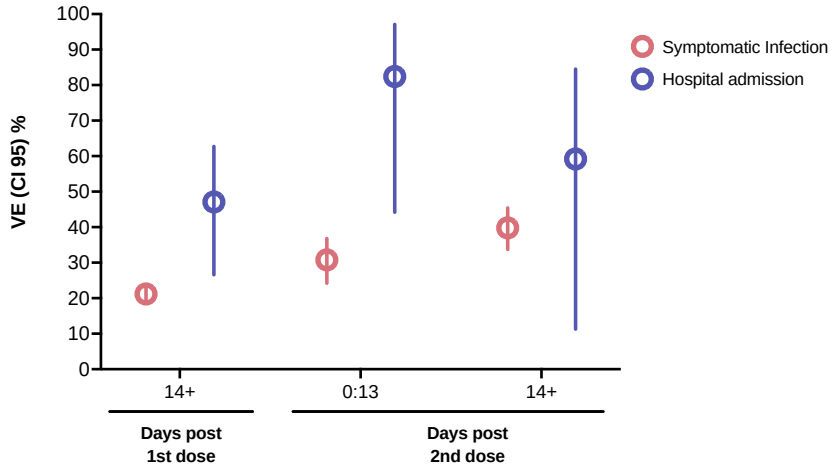


Fig. 1 | Vaccine Effectiveness for symptomatic infection and hospital admission among children aged 6–11 vaccinated with CoronaVac. The dots represent the adjusted vaccine effectiveness (VE;1- adjusted odds ratio) estimates (sample $n = 197{,}958$), with error bars indicating the corresponding 95% Wald's C.I. for symptomatic infection and Profile's likelihood C.I. for hospital admission. Red represents adjusted VE against symptomatic infection, and blue against hospital admission considering vaccination status (in days post first and second dose). The comparison group was the unvaccinated.

A more detailed description from our database can be found in the Supplementary Materials. In addition, we deterministically linked the data using the information provided by DATASUS from the Brazilian Ministry of Health. Dataset quality assessment and linkage details have been described before[18–21].

Study design

We used a test-negative design, which is a type of case-control study among the population tested, with controls selected from those who presented a negative test[22]. The study population comprised childrens aged 6–11 years with COVID-19-related symptoms in Brazil from January 21, 2022, to April 15, 2022, with a predominant circulation of the Omicron variant (>98% of sequenced viruses)[23]. We linked records of SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) and antigen tests to national vaccination and clinical records. Participants were symptomatic children with a sample collected within ten days of symptom onset. Cases of confirmed infection were those with a positive SARS-CoV-2 RT-PCR or antigen test, and control had a negative SARS-CoV-2 RT-PCR or antigen test. Additionally, we evaluated severe COVID-19 (hospital admission), defined as a positive test that occurred within 14 days before the hospitalisation date and up to four days after hospital admission, and death occurring within 28 days after a positive test.

We excluded: (1) individuals older than 11 years and younger than 6 years; (2) individuals who received vaccines other than CoronaVac; and (3) tests among asymptomatic people and tests referring to a symptom onset date after the notification date; (4) individuals whose time interval between the first and second doses was less than 14 days and received first dose before January 21, 2022; (5) negative test within 14 days of a previous negative test; (6) negative test followed by a positive test up to 7 days; (7) any test after a positive test up to 90 days, and (8) tests with missing information on age, sex, city of residence, sample collection, or first symptoms date; (9) any individual which received the third dose. Our exposure was vaccination status stratified by the time since the last dose on the date of sample collection, categorised as: unvaccinated and, for the vaccinated, grouped in periods (days) after each dose: first dose (0–13 days, and ≥14 days), second dose (0–13, ≥14 days). In addition, the following confounders were included in the model: age, gender, ethnicity, time (month), region of residence, socioeconomic position measured by quintile of deprivation (the Índice Brasileiro de Privação in Brazil)[24], previous SARS-CoV-2 infection (between 3–6 months or more six months ago), number of comorbidities commonly associated with COVID-19 illness. The odds ratio (OR) comparing the odds of vaccination between cases and controls and its associated 95% Confidence Interval (CI) were derived using logistic regression. VE was estimated as (1-OR)*100, obtained from an adjusted model including the described covariates, expressed as a percentage. All data processing and analyses were performed in R (version 4.1.1)[25], using the Tidyverse package[26]. Missing values relating to ethnicity were imputed using multiple imputations, as sensitivity analyses. For these analyses, we used the MICE package (version 1.16) with five imputations[27]. We conducted a logistic regression for rare events (ICU admission) using Firth's bias reduction method (Logistf package v. 1.24.1)[28].

We followed the RECORD reporting guidelines (Table S5)[29]. The statistical analysis plan (SAP) was published in https://vigivac.fiocruz.br/. The Brazilian National Commission in Research Ethics approved the research protocol (CONEP approval number 4.921.308) and (CAAE registration no. 50199321.9.0000.0040). CONEP waived the requirement for informed consent because we did not have access to identified data. The Brazilian Ministry of Health authorized the use of these data by the Vaccination Digital Vigilance (VigiVac) program under the data protection law which allows such a consent for public health research.

Data availability

Our statistical analysis plan is available at https://vigivac.fiocruz.br. Regarding Brazilian data availability, one of the study coordinators (M.B.-N.) signed a term of responsibility on using each database made available by the Ministry of Health (MoH). Each member of the research team signed a term of confidentiality before accessing the data. Data was manipulated in a secure computing environment, ensuring protection against data leakage. The Brazilian National Commission in Research Ethics approved the research protocol (CONEP approval no. 4.921.308). Our agreement with the MoH for accessing the databases patently denies authorization of access to a third party. Any information for assessing the databases must be addressed to the Brazilian MoH at https://datasus.saude.gov.br/, and requests can be addressed to datasus@saude.gov.br. In this study, we used anonymized secondary data following the Brazilian Personal Data Protection General Law, but it is vulnerable to re-identification by third parties as they contain dates of relevant health events regarding the same person. To protect the research participants' privacy, the approved Research Protocol (CONEP approval no. 4.921.308)

authorises the dissemination only of aggregated data, such as the data presented here.

Code availability

All code used in this study is publicly available at https://github.com/cidacslab/vigivac/tree/main/tnd_02.

References

1. Frenck, R. W. et al. Safety, immunogenicity, and efficacy of the BNT162b2 Covid-19 vaccine in adolescents. *N. Engl. J. Med.* **385**, 239–250 (2021).
2. Walter, E. B. et al. Evaluation of the BNT162b2 Covid-19 vaccine in children 5 to 11 years of age. *N. Engl. J. Med.* **386**, 35–46 (2022).
3. Center for Disease Control and Prevention. Multisystem inflammatory syndrome in children (MIS-C) associated with coronavirus disease 2019 (COVID-19). (2020). Available at: https://emergency.cdc.gov/han/2020/han00432.asp. (Accessed: 14th May 2020).
4. Stein, M. et al. Project Report The Burden of COVID-19 in children and its prevention by vaccination: a joint statement of the israeli pediatric association and the israeli society for pediatric infectious diseases. *Vaccines* **10**, 81 (2022).
5. Ministério da Saúde do Brasil. *Nota Técnica N°6/2022-Secovid/Gab/Secovid/Ms*. (2022).
6. Jara, A. et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in children and adolescents: a large- scale observational study. *SSRN* (2022).
7. Jara, A. et al. Effectiveness of CoronaVac in children 3 to 5 years during the omicron SARS-CoV-2 outbreak. *Nat. Med.* **28**, 1377–1380 (2022).
8. Florentino, P. T. V. et al. Vaccine effectiveness of two-dose BNT162b2 over time against COVID-19 symptomatic infection and severe cases among adolescents: test negative design case control studies in Brazil and Scotland. *The Lancet Infectious Diseases*. 1–23 (2022).
9. Andrews, N. et al. Covid-19 vaccine effectiveness against the Omicron (B.1.1.529) variant. *N. Engl. J. Med.* **386**, 1532–1546 (2022).
10. Buchan, S. A., Nguyen, L., Wilson, S. E., Kitchen, S. A. & Kwong, J. Vaccine effectiveness of BNT162b2 against Omicron and Delta outcomes in adolescents. *medRxiv* 2022.04.07.22273319 (2022).
11. Veneti, L. et al. Vaccine effectiveness with BNT162b2 (Comirnaty, Pfizer-BioNTtech) vaccine against reported SARS-CoV-2 delta and omicron infection among adolescents, Norway, August 2021 to January 2022. *SSRN Electron. J.* **2**, 1–11 (2022).
12. Dorabawila, V. et al. Effectiveness of the BNT162b2 vaccine among children 5–11 and 12–17 years in New York after the emergence of the Omicron Variant. *medRxiv* 2022.02.25.22271454 (2022).
13. Sacco, C. et al. Articles Effectiveness of BNT162b2 vaccine against SARS-CoV-2 infection and severe COVID-19 in children aged 5–11 years in Italy: a retrospective analysis of January-April, 2022. *Lancet* **2**, 1–7 (2022).
14. Vandenbroucke, J. & Pearce, N. Test-negative designs differences and commonalities with other case–control studies with "other patient" controls. *Epidemiology* **30**, 838–844 (2019).
15. Dawood, F. S., Porucznik, C. A., Veguilla, V. & Al, E. Incidence rates, household infection risk, and clinical characteristics of SARS-CoV-2 infection among children and adults in Utah and New York City, New York. *Jama Pediatrics* **176**, 59–67 (2022).
16. Mensah, A. A. et al. Risk of SARS-CoV-2 reinfections in children: a prospective national surveillance study between January, 2020, and July, 2021, in England. *Lancet Child Adolesc. Health* **6**, 384–392 (2022).
17. Lipsitch, M., Goldstein, E., Ray, G. T. & Fireman, B. Depletion-of-susceptibles bias in influenza vaccine waning studies: how to ensure robust results. *Epidemiol. Infect.* **147**, e306 (2019).
18. Katikireddi, S. V. et al. Two-dose ChAdOx1 nCoV-19 vaccine protection against COVID-19 hospital admissions and deaths over time: a retrospective, population-based cohort study in Scotland and Brazil. *Lancet* **399**, 25–35 (2022).
19. Cerqueira-Silva, T. et al. Vaccine effectiveness of heterologous CoronaVac plus BNT162b2 in Brazil. *Nat. Med.* **28**, 838–843 (2022).
20. Cerqueira-Silva, T. et al. Vaccination plus previous infection: protection during the omicron wave in Brazil. *Lancet Infect. Dis.* **22**, 1–2 (2022).
21. Cerqueira-Silva, T. et al. Effectiveness of CoronaVac, ChAdOx1 nCoV-19, BNT162b2, and Ad26.COV2.S among individuals with previous SARS-CoV-2 infection in Brazil: a test-negative, case-control study. *Lancet Infect. Dis.* **22**, 791–801 (2022).
22. Vandenbroucke, J. P., Brickley, E. B., Vandenbroucke-Grauls, C. M. J. E. & Pearce, N. A test-negative design with additional population controls can be used to rapidly study causes of the SARS-COV-2 epidemic. *Epidemiology* **31**, 836–843 (2020).
23. Fiocruz. GISAID. *Rede Genômica* (2020). Available at: http://www.genomahcov.fiocruz.br/#. (Accessed: 15th May 2022)
24. Allik, M. et al. Small-area Deprivation Measure for Brazil: Data Documentation. *University of Glasgow* (2020). Available at: https://researchdata.gla.ac.uk/980/. (Accessed: 15th May 2020).
25. Vienna, A. R. Core Team. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing;* (2021).
26. Wickham, H. et al. Welcome to the Tidyverse Tidyverse package. *J. Open Source Softw.* **4**, 1–6 (2019).
27. van Buuren, S. & Groothuis-Oudshoorn, K. mice: Multivariate imputation by chained equations in R. *J. Stat. Softw.* **45**, 1–67 (2011).
28. Firth, D. "Bias reduction of maximum likelihood estimates.". *Biometrika* **82**, 667 (1995).
29. Benchimol, E. I. et al. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Med.* **12**, 1–22 (2015).

Acknowledgements

This study is part of the Fiocruz VigiVac programme and the authors acknowledge DATASUS for its diligent work in providing the unidentified Brazilian databases. This study was partially supported by a donation from the "Fazer o bem faz bem" program, from JBS, S.A, GLW, MLB, VB and M.B-N. are research fellows from the Brazilian National Research Council. M.B-N. acknowledges Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB)–Grant PNX0008/2014/ Fapesb, Edital 08/2014–Programa de Apoio a Núcleos de Excelência. G.L.W. acknowledges Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (E-26/210.180/2020). ESP is funded by the Wellcome Trust [Grant number 213589/Z/18/Z]. E.P.S. is funded by the Wellcome Trust [Grant number 213589/Z/18/Z]. The funders had no role in study design, data collection, data analysis, data interpretation, the report's writing, or decision to publish. For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript (AAM) version arising from this submission.

Author contributions

E.S.P., M.L.B. and M.B.-N. conceived the idea for the study. All authors contributed to the study design, with P.T.V.F., E.S.P., A.G.J. and T.C.-S. drafting the statistical analysis plan. P.T.V.F. conducted the statistical analysis, E.S.P. checked the analysis code. P.T.V.F., J.B.S.J., T.C.-S. and V.dAO. had access to individual-level data for Brazil and performed data linkage. M.B.-N., V.dAO. and M.L.B. organised the data linkage and secured funding. E.S.P., P.T.V.F. and F.J.O.A. wrote the initial draft of the manuscript. E.S.P., L.R., G.L.W., G.O.P., M.L.B., V.B., N.P., and M.B.-N. critically revised the manuscript. PTVF and VdAO accessed and verified the data and analyses. All authors critically revised the manuscript and approved the final version for submission.

Competing interests

M.B.-N. reports grants from the Fazer o bem faz bem program from JBS. S.A. V.dA.O., V.B., M.L.B., and M.B.-N. are employees of Fiocruz, a federal public institution, which manufactures Vaxzevria in Brazil, through a full technology transfer agreement with AstraZeneca. Fiocruz allocates all its manufactured products to the Ministry of Health for public health use. The remaining authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-32524-5.

Correspondence and requests for materials should be addressed to Pilar T. V. Florentino.

Peer review information *Nature Communications* thanks Nimalan Arinaminpathy and Alejandro Jara for their contribution to the peer review of this work. Peer reviewer reports are available.

6.3. CoronaVac induz anticorpos em mais de 90% das crianças, incluindo aquelas com comorbidades, mostra estudo chileno

Uma pesquisa do Chile voltou a comprovar a imunogenicidade da CoronaVac em crianças e adolescentes, com soroconversão atingindo 91,8% um mês após a segunda dose. A vacina teve um desempenho ainda melhor naqueles com comorbidades, como obesidade, chegando a 97,4% nesse grupo. O estudo foi publicado no International Journal of Infectious Diseases e conduzido pelos Ministérios de Saúde e de Educação do país e pela Faculdade de Medicina da Universidade do Chile. O país aplica a CoronaVac em crianças a partir dos 3 anos desde dezembro de 2021 e, no final de agosto, aprovou a aplicação do imunizante a partir dos 6 meses.

Os cientistas avaliaram a soroconversão de anticorpos IgG em cerca de 2 mil crianças e adolescentes de 6 a 18 anos, estudantes de 24 escolas localizadas nas três regiões mais populosas do Chile. No grupo analisado, 173 crianças tinham comorbidades como obesidade, doença pulmonar crônica, doenças cardiovasculares, hipertensão, diabetes e câncer.

A porcentagem de crianças que produziram anticorpos contra o SARS-CoV-2 (soroconversão) foi de 91,8% um mês após a segunda dose. Analisando os indivíduos separadamente de acordo com faixa etária, sexo, localização e presença de comorbidades, a soroconversão se manteve alta, acima de 85%, na maior parte dos grupos.

A vacina, recentemente aprovada no Brasil para a faixa etária de 3 a 5 anos, e que tem sido aplicada desde janeiro no grupo de 6 a 17, já teve a sua segurança comprovada em diversas pesquisas. Outro estudo, também do Chile, mostrou que a CoronaVac é o imunizante com a menor taxa de eventos adversos dentre aqueles que são aplicados em crianças no país, com apenas 10,67 notificações a cada 100 mil doses – 0,01% do total de doses aplicadas. A vacina também tem a menor taxa de eventos adversos nos adultos.

A eficiência da CoronaVac contra casos graves também foi demonstrada em estudos de mundo real. No Brasil, uma pesquisa recente da Fundação Oswaldo Cruz (Fiocruz) registrou uma efetividade de 59,2% contra hospitalizações por Covid-19 no público de 6 a 17 anos. No Chile, em crianças de 3 a 5 anos, a efetividade foi de 69% contra internação em Unidade de Terapia Intensiva (UTI) e 64,6% contra hospitalização, mesmo durante o surto da ômicron.

Publicado em: 24/5/2022

International Journal of Infectious Diseases 121 (2022) 89–91

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Short Communication

SARS-COV-2 IgG positivity in vaccinated and non-vaccinated Chilean children: a national cross-sectional study in schools

Juan P. Torres[1], Denis Sauré[2,4], Leonardo J. Basso[3,4], Marcela Zuñiga[5], Andre Cazor[6], Miguel O'Ryan[7,*]

[1] Department of Pediatrics, Hospital Calvo Mackenna, Facultad de Medicina, Universidad de Chile, Antonio Varas 360, Santiago, Chile
[2] Department Industrial Engineering, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Beauchef 850, Santiago, Chile
[3] Department of Civil Engineering, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Beauchef 850, Santiago, Chile
[4] Instituto Sistemas Complejos de Ingeniería, República 695, Santiago, Chile
[5] Ministerio de Salud, Gobierno de Chile, Santiago, Chile
[6] Ministerio de Educación, Gobierno de Chile, Santiago, Chile
[7] Program of Microbiology and Micology, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Independencia 1027, Santiago, Chile

ARTICLE INFO

Article history:
Received 27 February 2022
Revised 14 April 2022
Accepted 19 April 2022

Introduction

COVID-19 vaccination of children is gaining global support (Committee on Infectious Diseases, 2022), and data on immunogenicity and efficacy/effectiveness are increasing (Walter et al., 2022; Frenck et al., 2021; Han et al., 2021). Chile has rapidly advanced in a national vaccination campaign for children: as of February 17, 2022, 79% of children aged 3–17 years have been fully vaccinated (Ministerio de Salud Chile, 2022). Children aged 12–17 years have been vaccinated since June 22, 2021, with the mRNA Pfizer/BioNTech vaccine, followed weeks later by children aged 6–11 years, who received the inactivated Sinovac vaccine. We previously reported a national COVID-19 IgG seropositivity study in adults vaccinated with either vaccine that demonstrated the utility of large cross-sectional immunologic surveys using lateral flow tests (LFTs) (Sauré et al., 2022). In this study, we reported IgG seropositivity in vaccinated and non-vaccinated Chilean school-aged children who received the inactivated vaccine from Sinovac (CoronaVac) or the mRNA vaccine from Pfizer/BioNTech (BNT162b2) within 1–20 weeks before sample collection, or no vaccine. Data on IgG seropositivity among vaccinated children with inactivated as compared with mRNA vaccines are currently non-existent and can provide important information for decision-makers worldwide.

Methods

We performed SARS-CoV-2 IgG testing using the OnSite (CTK Biotech Inc, Poway, CA, US) LFT. This was the same LFT as the one used in adults (Sauré et al., 2022), with reported sensitivity and specificity of 96.7% and 98.1%, respectively (CTK Biotech, 2021). In conjunction with the Chilean Ministries of Education and Health, 24 schools located in the three most populated regions in Chile were invited to take part in the study. Briefly, all parent/children pairs were invited to participate through a letter sent by school authorities. Accepting parents signed informed consent, and children aged >8 years an assent. Children of every accepting parent were tested. Trained staff in each school obtained basic information from the parent/caregiver of the child participant, including type of vaccine and vaccination dates, age, gender, country of origin, general medical history, previous COVID-19 IgG or polymerase chain reaction testing, home address and usual transportation method to school. A finger-prick blood sample was obtained from children as previously described (Sauré et al., 2022). Tests were read on-site and results (positive, negative, or not conclusive) and surveillance data were instantly uploaded through a web interface to a database harbored at the Instituto Sistemas Complejos de Ingeniería, as in previous reports (Sauré et al., 2022). The study was approved by the Comité de Ética de Investigación en Seres Humanos (Universidad de Chile, Santiago, Chile).

* Corresponding author: Miguel O'Ryan, Programa de Microbiologia e Inmunologia, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Av. Independencia 1027, Independencia, Santiago, Chile,+569 61401237
E-mail address: moryan@uchile.cl (M. O'Ryan).

https://doi.org/10.1016/j.ijid.2022.04.039

Table 1
Covid-19 IgG positivity according to population characteristics and vaccine received[a].

Characteristics	Total		Unvaccinated		Sinovac		Pfizer	
	n/N	IgG positivity (95% CI)	n/N	IgG positivity (95% CI)	n/N	IgG positivity (95% CI)	n/N	IgG positivity (95% CI)
Age range								
6–11 years	837/1033	81.0% (78.6%, 83.4%)	25/90	27.8% (18.5%, 37.0%)	792/920	86.1% (83.9%, 88.3%)	20/23	87.0% (73.2%, 100%)
12–18 years	1136/1269	89.5% (87.8%, 91.2%)	7/31	22.6% (7.9%, 37.3%)	505/591	85.4% (82.6%, 88.3%)	624/647	96.4% (95.0%, 97.9%)
Gender								
Male	866/1001	86.5% (84.4%, 88.6%)	15/62	24.2% (13.5%, 34.9%)	598/678	88.2% (85.8%, 90.6%)	253/261	96.9% (94.8%, 99.0%)
Female	1107/1301	85.1% (83.2%, 87.0%)	17/59	28.8% (17.3%, 40.4%)	699/833	83.9% (81.4%, 86.4%)	391/409	95.6% (93.6%, 97.6%)
Region								
Metropolitan	1301/1459	89.2% (87.6%, 90.8%)	19/72	26.4% (16.2%, 36.6%)	920/1021	90.1% (88.3%, 91.9%)	362/366	98.9% (97.8%, 100%)
Valparaíso	374/461	81.1% (77.6%, 84.7%)	12/36	33.3% (17.9%, 48.7%)	238/292	81.5% (77.1%, 86.0%)	124/133	93.2% (89.0%, 97.5%)
Biobío	298/381	78.2% (74.1%, 82.4%)	1/13	7.7% (0%, 22.2%)	139/197	70.6% (64.2%, 76.9%)	158/171	92.4% (88.4%, 96.4%)
Prev. pos. PCR[b]	35/45	77.8% (65.6%, 89.9%)	3/6	50.0% (10.0%, 90.0%)	20/27	74.1% (57.5%, 90.6%)	12/12	100% (100%, 100%)
Comorbidities								
Obesity	50/56	89.3% (81.2%, 97.4%)	1/6	16.7% (0%, 46.5%)	38/39	97.4% (92.5%, 100%)	11/11	100% (100%, 100%)
Chronic pulmonary disease	82/94	87.2% (80.5%, 94.0%)	1/4	25.0% (0%, 67.4%)	33/40	82.5% (70.7%, 94.3%)	48/50	96.0% (90.6%, 100%)
Cardiovascular	13/14	92.9% (79.4%, 100%)	0/0	-	6/7	85.7% (59.8%, 100%)	7/7	100% (100%, 100%)
Other[c]	8/9	88.9% (68.4%,100%)	0/0	-	0/0	-	8/9	88.9% (68.4%, 100%)
None identified	1820/2129	85.5% (84.0%, 87.0%)	30/111	27.0% (18.8%, 35.3%)	1220/1425	85.6% (83.8%, 87.4%)	570/593	96.1% (94.6%, 97.7%)
Total	**1973/2302**	85.7% (84.3%, 87.1%)	**32/121**	26.4% (18.6%, 34.3%)	**1297/1511**	85.8% (84.1%, 87.6%)	**644/670**	96.1% (94.7%, 97.6%)

CI, confidence interval; PCR, polymerase chain reaction.
[a] The data exclude participants with incomplete information (n=6), inconsistent vaccination status information (n=86), region other than those listed (n=1) and those vaccinated with vaccines other than Sinovac or Pfizer (n=11)
[b] Positive PCR previously obtained
[c] Includes four cases of hypertension, four cases of diabetes and one case of cancer.

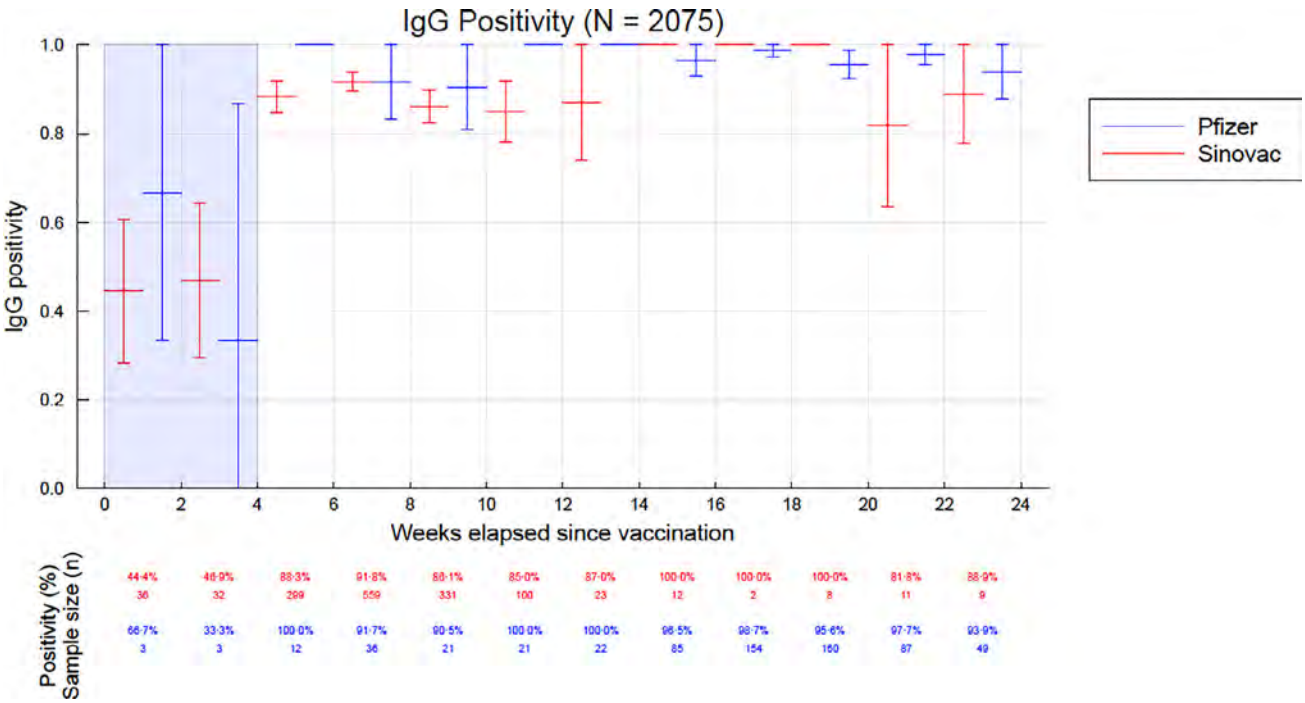


Fig. 1. Seropositivity one to four weeks after first dose (light blue-shaded region) or after second dose for recipients of Sinovac or Pfizer vaccines with no prior positive PCR result.

Results

As of December 24, 2021, a total of 2302 children have been included, as described in Table 1. Whereas most Sinovac recipients were aged 6–11 years (920), Pfizer/BioNTech recipients were almost exclusively aged 12–18 years (647). IgG positivity was significantly higher in Pfizer than in Sinovac recipients for all study variables except comorbidities (Table 1). In 670 children receiving the Pfizer/BioNTech vaccine, seropositivity was 91.7% three to four weeks after the second dose, with figures above 90% by 20 weeks after full vaccination (Fig. 1). In 1506 children receiving Sinovac, seropositivity was 91.8 % three to four weeks after the second dose, with a declining trend thereafter (Fig. 1).

Discussion

In school-aged Chilean children, SARS-CoV-2 IgG seropositivity surpassed 90% two weeks after the administration of a sec-

ond dose in the case of the inactivated vaccine (Sinovac), and up to 10 weeks after administering a second dose in the case of the mRNA vaccine (Pfizer/BioNTech). Compared with the adult population (Sauré et al., 2022), children showed a slightly weaker response to the mRNA vaccine and a slightly stronger response to the inactivated vaccine in terms of the overall proportion of seropositive individuals in the short-term period after vaccination. Nevertheless, in the case of adults, seropositivity in the inactivated vaccine recipients declines over time, suggesting that a booster dose will most likely be required for children; however, by 22–24 weeks after immunization, we reported a small sample size for the inactivated vaccine. LFTs do not differentiate IgG responses due to vaccination vs infection, which may have influenced some of the responses observed; positivity in a small number of non-vaccinated children reached 27%. Self-reporting of child characteristics reduces robustness for the comparison of comorbidities.

Chile was one of the first Western countries to begin vaccinating children (Ministerio de Salud 2021), a decision that may be relevant given the scenario of circulation of more transmissible variants. With the Omicron variant, SARS-CoV-2 infections and hospitalizations reached high levels in children, but severe clinical outcomes were less frequent than with the Delta variant in this population (Wang et al., 2022). The impact of the COVID-19 vaccines on protection against infection and especially severe disease has yet to be elucidated in children. However, immunization of children could have an impact on both direct and indirect effects of SARS-CoV-2 infection, favoring school attendance, mental health and cognitive learning, especially in vulnerable children (Fore, 2020).

Declaration of Competing Interest

The authors have no conflicts of interest relevant to this article to disclose.

Ethical approval

This study was approved by the Ethics Committee for Clinical Investigation in Humans from the Faculty of Medicine, Universidad de Chile.

Funding source

This study was supported by funds provided by the Ministerio de Salud, Gobierno de Chile. The Ministerio de Salud had no role in designing the study, provided the lateral flow tests used in the study, and funded filed work.

Acknowledgments

All authors declare no conflict of interest. This work was partially supported by a grant from the Instituto Sistemas Complejos de Ingeniería (ANID PIA AFB 180003). Field work and lateral flow tests were funded by the Subsecretaría de Redes Asistenciales, Ministerio de Salud, Chile. We appreciate the important support of all the personnel of the Ministry of Education of Chile in each school of the study.

References

Committee on Infectious Diseases. COVID-19 vaccines in children and adolescents. Pediatrics 2022;149 [E-pub ahead of print].

CTK Biotech.com. Instructions for users, 2021, https://drive.google.com/file/d/1ATIHN4yKm_X9GPgJZER46e9YSJZfuO6R/view?usp=sharing; (accessed December 10th, 2021).

Fore HH. A wake-up call: COVID-19 and its impact on children's health and wellbeing. Lancet Glob Health 2020;8:e861–2.

Frenck Jr RW, Klein NP, Kitchin N, Gurtman A, Absalon J, Lockhart S, et al. Safety, immunogenicity, and efficacy of the BNT162b2 Covid-19 vaccine in adolescents. N Engl J Med 2021;385:239–50.

Han B, Song Y, Li C, Yang W, Ma Q, Jiang Z, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021;21:1645–53.

Ministerio de Salud. Chile 2021, https://www.minsal.cl/autoridades-dan-inicio-a-la-vacunacion-contra-el-covid-19-en-ninos-desde-los-6-anos/; (accessed January 3rd, 2022)- in spanish.

Ministerio de Salud. Chile 2022. Departamento de Estadísticas e Información de Salud, https://informesdeis.minsal.cl/SASVisualAnalytics/?reportUri=%2Freports%2Freports%2F9037e283-1278-422c-84c4-16e42a7026c8&sectionIndex=1&sso_guest=true&sas-welcome=false; (accessed February 19, 2022).

Sauré D, O'Ryan M, Torres JP, Zuñiga M, Santelices E, Basso LJ. Dynamic IgG seropositivity after rollout of CoronaVac and BNT162b2 COVID-19 vaccines in Chile: a sentinel surveillance study. Lancet Infect Dis 2022;22:56–63.

Walter EB, Talaat KR, Sabharwal C, Gurtman A, Lockhart S, Paulsen GC, et al. Evaluation of the BNT162b2 Covid-19 vaccine in children 5 to 11 years of age. N Engl J Med 2022;386:35–46.

Wang L, Berger NA, Kaelber DC, Davis PB, Volkow ND, Xu R. Incidence rates and clinical outcomes of SARS-CoV-2 infection with the omicron and Delta variants in children younger than 5 years in the US. JAMA Pediatr 2022 [E-pub ahead of print].

6.4. CoronaVac tem a menor taxa de eventos adversos entre vacinas contra Covid-19 disponíveis para crianças e adolescentes no Chile

Dados da farmacovigilância do governo chileno indicam que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, é o imunizante com as menores taxas de eventos adversos em crianças e adolescentes, entre três a 17 anos, que receberam vacinas contra Covid-19 no país. A CoronaVac é o imunizante mais seguro tanto em notificações gerais, quanto comparado à outra vacina e entre pessoas de idades e sexos diferentes, segundo dados do 4° Informe Estatístico "ESAVI associados a administração de vacinas SARS-CoV-2 no Chile na população pediátrica e adolescentes", do Ministério da Saúde local.

Entre 1° de março de 2021 e 26 de fevereiro de 2022 foram administradas 6,9 milhões de doses de vacinas contra a Covid-19 em menores de 18 anos no Chile. Destas, 4,9 milhões de doses eram da CoronaVac e 2 milhões de doses da Comirnaty, vacina da Pfizer/BioNTech. No mesmo período foram notificados pela farmacovigilância chilena 868 Eventos Supostamente Atribuíveis à Vacinação ou Imunização (ESAVIs) entre pessoas de três a 17 anos que tomaram as duas vacinas, com taxas menores de eventos adversos entre os que receberam a CoronaVac.

ESAVI é uma condição de saúde desfavorável, não intencional, que pode ser um sintoma, um achado laboratorial ou uma doença, ocorrido após a vacinação (administração da vacina) ou imunização (geração de resposta imune), segundo a Organização Pan-Americana da Saúde (Opas).

"O total de notificações recebidas associadas à CoronaVac foi de 520, o que corresponde a 0,01% do total de doses administradas e uma taxa de notificação de 10,67 notificações a cada 100 mil doses", descreve o relatório.

A vacina de RNA mensageiro (Pfizer), por sua vez, demonstrou uma taxa de 15,35 notificações de ESAVIs a cada 100 mil doses da vacina aplicadas. A taxa é maior do que entre os que tomaram a CoronaVac, mesmo com um número menor de doses administradas na faixa etária analisada.

Estes dados comprovam que a CoronaVac é uma vacina com raros efeitos adversos no público infantojuvenil e quando eles aparecem costumam se resumir a dor no local da aplicação na maioria dos relatos. Tanto que das 520 notificações, 456 foram de eventos considerados não graves, ou seja, cujos sintomas desaparecem sem necessidade de tratamento sintomático e hospitalização e que não colocam em risco a vida, em definição da Opas.

O Ministério da Saúde chileno liberou o uso da CoronaVac em crianças a partir de seis anos em 6 de setembro de 2021 e em crianças a partir de 3 anos em 30 de novembro de 2021. A vacina de RNA mensageiro é usada

no país em pessoas a partir de 5 anos desde 21 de dezembro de 2021.

Os dados foram colhidos pelo Subdepartamento de Farmacovigilância (SDFV, na sigla em espanhol), do Instituto de Saúde Pública do Chile (ISP), ligado ao Ministério da Saúde local, que notifica e investiga se os ESAVIs têm ou não relação com a vacinação.

CoronaVac mantém segurança entre as doses

As notificações de eventos adversos entre a primeira e a segunda dose também se mostraram menores em quem tomou a CoronaVac. Segundo o relatório chileno, a primeira dose de CoronaVac foi associada a 12,92 notificações e a segunda dose a 4,24 notificações de ESAVIs para cada 100 mil doses aplicadas. Entre os que tomaram a vacina de RNA mensageiro, foram 16,67 notificações de ESAVIs na primeira dose e 10,41 na segunda dose a cada 100 mil doses aplicadas do imunizante.

Diferenças por faixa etária e sexo

As taxas de eventos adversos também são menores entre meninos e meninas que tomaram a CoronaVac em comparação com a outra vacina disponível. De acordo com o relatório, a taxa de notificação de ESAVIs entre meninos de 12 a 17 anos que tomaram CoronaVac é de 9,45 notificações para cada 100 mil doses, enquanto a taxa em meninos da mesma faixa etária, que tomaram a vacina de RNA mensageiro, é de 14,57 para cada 100 mil doses aplicadas. Entre as meninas de 12 a 17 anos que tomaram CoronaVac, a taxa de ESAVIs foi de 10,58 para cada 100 mil doses, enquanto entre as meninas da mesma faixa etária que tomaram a outra vacina foi de 15,46 notificações a cada 100 mil doses.

Quanto à faixa etária de 3 a 11 anos, os dados de eventos adversos foram computados apenas em relação à CoronaVac. Entre meninos desta faixa etária, a taxa foi de 10,76 notificações de ESAVIs para 100 mil doses e 11,06 notificações para mil 100 doses entre as meninas desta faixa etária.

Efeitos adversos mais comuns

Segundo o documento, dor no local da injeção corresponde a 3,26 notificações a cada 100 mil doses de CoronaVac aplicadas, na média geral. Prurido (1,99 notificações a cada 100 mil doses), cefaleia (1,60 a cada 100 mil doses), vômitos (1,54 notificações a cada 100 mil doses), urticária (1,42), febre (1,07) e náuseas (1,05) são os outros eventos mais frequentes. Todos os dados são menos incidentes do que os relatados nas vacinas de RNA mensageiro.

Publicado em: 28/6/2022

Artigo

Instituto de Salud Pública
Ministerio de Salud
Gobierno de Chile

Cuarto Informe Estadístico

"ESAVI asociados a la administración de vacunas SARS-CoV-2 en Chile"

En población pediátrica y adolescentes

Periodo: 01 de marzo 2021 al 26 de febrero de 2022

Mayo 2022

Santiago, Chile

Departamento Agencia Nacional de Medicamentos

Índice

Vacunas SARS-CoV-2 Autorizadas y utilizadas en Chile en población menor de 18 años

En Chile, debido a la pandemia ocasionada por el virus SARS-CoV-2, se ha aprobado el uso de emergencia de dos vacunas en pacientes pediátricos y adolescentes, contribuyendo de esta manera, a las estrategias implementadas en el país para el control y mitigación de la propagación de este virus, con el objetivo de disminuir el riesgo de contagio de COVID-19 y prevenir los síntomas graves de dicha enfermedad. En la tabla 1, se resume la información de las vacunas SARS-CoV-2 utilizadas en nuestro país para el grupo etario menor a 18 años.

Tabla 1. Vacunas SARS-CoV-2 autorizadas y en uso a la fecha en Chile en población menor de 18 años.

Fabricante	Fecha de autorización Vacunas SARS-CoV-2 en población menor de 18 años	Indicaciones aprobadas al 26 de febrero de 2022
Pfizer - BioNTech	15 de diciembre de 2020	Inmunización activa contra la enfermedad COVID-19 causada por el virus SARS-CoV-2, en personas desde los 16 años.
	31 de mayo de 2021	Inmunización activa contra la enfermedad COVID-19 causada por el virus SARS-CoV-2, en personas desde los 12 años.
	21 de diciembre de 2021	Inmunización activa contra la enfermedad COVID-19 causada por el virus SARS-CoV-2, en personas desde los 5 años.
Sinovac Life Sciences	06 de septiembre de 2021	Inmunización activa contra la enfermedad COVID-19 causada por el virus SARS-CoV-2, en personas desde los 6 años.
	30 de noviembre de 2021	Inmunización activa contra la enfermedad COVID-19 causada por el virus SARS-CoV-2, en personas desde los 3 años.

Nota: Se excluyen las siguientes vacunas SARS-CoV-2: Moderna, que, si bien el 03 de febrero 2022 se aprobó su importación con indicación de uso en personas desde los 12 años, aún no se utiliza en el país en este grupo etario, en el periodo comprendido en este informe.

Dosis administradas de vacunas SARS-CoV-2

Desde la implementación de la campaña de inmunización con vacunas SARS-CoV-2 en Chile, hasta el 26 de febrero de 2022, se han administrado 6.946.593 dosis de vacunas SARS-CoV-2 a menores de 18 años[1]. La distribución de cada vacuna SARS-CoV-2, según laboratorio fabricante, se muestra en la tabla 2.

Tabla 2. Número de dosis administradas de vacunas SARS-CoV-2 distribuidas por laboratorio fabricante, periodo 01 marzo de 2021 a 26 febrero de 2022 en menores de 18 años.

Laboratorio fabricante Vacuna SARS-CoV-2, Plataforma	1° dosis	2° dosis	1°Refuerzo	2°Refuerzo	Total
Pfizer-BioNTech, ARN mensajero	779.619	739.701	552.523	151	2.071.994
Sinovac, Inactivada	2.585.174	2.289.215	207	3	4.874.599
Total	3.364.793	3.028.916	552.730	154	6.946.593

ESAVI reportados al SDFV

El Subdepartamento Farmacovigilancia (SDFV) del Instituto de Salud Pública de Chile (ISP), recibe las notificaciones de Eventos Supuestamente Atribuibles a la Vacunación e Inmunización (ESAVI) a través de 4 vías: sistemas de notificación electrónica ESAVI-EPRO, REDRAM y NOTI-RAM ESAVI, y el formulario manual recibido por correo electrónico.

El presente informe abarca los datos recogidos de las notificaciones de ESAVI de las vacunas SARS-CoV-2 recibidas en el SDFV en el periodo definido anteriormente. Es importante señalar que estas notificaciones fueron recopiladas mediante el método de vigilancia pasiva e informadas por profesionales de la salud. Los ESAVI no se pueden considerar relacionados a las vacunas, hasta que no se confirme una relación causal con su administración. Esta evaluación se lleva a cabo de manera rutinaria por la sección Farmacovigilancia de Vacunas del SDFV, así como por el equipo de Farmacovigilancia de Vacunas del SDFV. Este equipo está compuesto por un Comité de Expertos Externos al ISP, integrantes del Programa Nacional de Inmunizaciones del MINSAL, y profesionales del SDFV.

[1] Según información obtenida desde el Departamento de Inmunizaciones, MINSAL

En el periodo de estudio se recibió un total de 868 notificaciones de ESAVI, lo que corresponde al 0,01% del total de dosis administradas y a una tasa total de 12,50 notificaciones por cada 100.000 dosis administradas, en menores de 18 años. De ellas, 107 corresponden a eventos serios, lo que representa un 0,002% del total de dosis administradas y a una tasa de 1,54 notificaciones por 100.000 dosis administradas.

Distribución de ESAVI vacunas SARS-CoV-2, por laboratorio fabricante

De un total de 868 notificaciones recibidas en el SDFV, podemos observar, en la tabla 3, la distribución de notificaciones según laboratorio fabricante. La vacuna con un mayor número de notificaciones corresponde a la vacuna SARS-CoV-2 Sinovac, con 520 notificaciones, que equivalen al 59,91% del total de notificaciones recibidas, sin embargo, al calcular las tasas por cada 100.000 dosis administradas, se evidencia que presenta una menor tasa de notificación en comparación con la vacuna SARS-CoV-2 Pfizer-BioNTech, con solo 10,67 notificaciones por cada 100.000 dosis administradas. En cuanto a la vacuna SARS-CoV-2 Pfizer-BioNTech, es la que posee la mayor tasa de notificación, con 15,35 notificaciones por cada 100.000 dosis administradas, lo que corresponde a 318 notificaciones, que equivale al 36,64% del total de notificaciones recibidas en el SDFV. Ambas tasas no difieren en gran medida, encontrándose en el mismo orden de magnitud.

Tabla 3. Distribución de ESAVI, según laboratorio fabricante, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

Vacuna SARS-CoV-2 Laboratorio fabricante	Número de notificaciones	Porcentaje respecto al total de notificaciones	Tasa de notificaciones cada 100.000 dosis administradas
Pfizer-BioNTech	318	36,64	15,35
Sinovac	520	59,90	10,67
No señala	30	3,46	-
Total	868	100	12,50

Distribución de ESAVI serios de vacunas SARS-CoV-2, por laboratorio fabricante

De un total de 868 notificaciones de ESAVI recibidas en el SDFV, 107 de ellas se clasificaron preliminarmente como serias[2], lo que equivale al 12,33% del total y a una tasa de 1,54 por cada 100.000 dosis administradas. En la tabla 4, se observa que la vacuna SARS-CoV-2 Pfizer-BioNTech presentó una tasa de 2,08 ESAVI serios por cada 100.000 dosis administradas, que equivale al 40,19% del total de las notificaciones serias, mientras que la vacuna SARS-CoV-2 Sinovac presentó una tasa de 1,31 por cada 100.000 dosis administradas, que equivale al 59,81% de las notificaciones serias reportadas. Nuevamente, ambas tasas se encuentran en el mismo orden de magnitud.

[2] Las notificaciones que se clasifican como serias son aquellas que amenazan la vida, causan hospitalización o la prolongan, resultan en incapacidad persistente o permanente, o resultan en la muerte del paciente.

Tabla 4. Distribución de ESAVI serios según laboratorio fabricante, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

Vacuna SARS-CoV-2 Laboratorio fabricante	Número de notificaciones serias	Porcentaje de notificaciones serias	Tasa de notificaciones cada 100.000 dosis administradas
Pfizer-BioNTech	43	40,19	2,08
Sinovac	64	59,81	1,31
Total	107	100,00	1,54

Distribución de ESAVI totales, por laboratorio fabricante y número de dosis administradas

En la figura 1, se observa la distribución de la tasa de notificaciones según el laboratorio fabricante y el número de dosis administrada del esquema primario (1° y 2° dosis), además de la dosis de refuerzo para la vacuna SARS-CoV-2 Pfizer-BioNTech. Para esta última, la tasa más elevada se presentó en la 1° dosis, con 16,67 por cada 100.000 dosis administradas, al igual que para la vacuna SARS-CoV-2 Sinovac, para la cual, sin embargo, se obtuvo una tasa más baja, de 12,92 notificaciones por cada 100.000 dosis administradas. El cálculo de la tasa de notificaciones con la 1° dosis de refuerzo para la vacuna SARS-CoV-2 Sinovac no fue incluido, dado el bajo número de personas que recibieron esta dosis durante el periodo de estudio, por lo que no se puede hacer una comparación entre ambas vacunas.

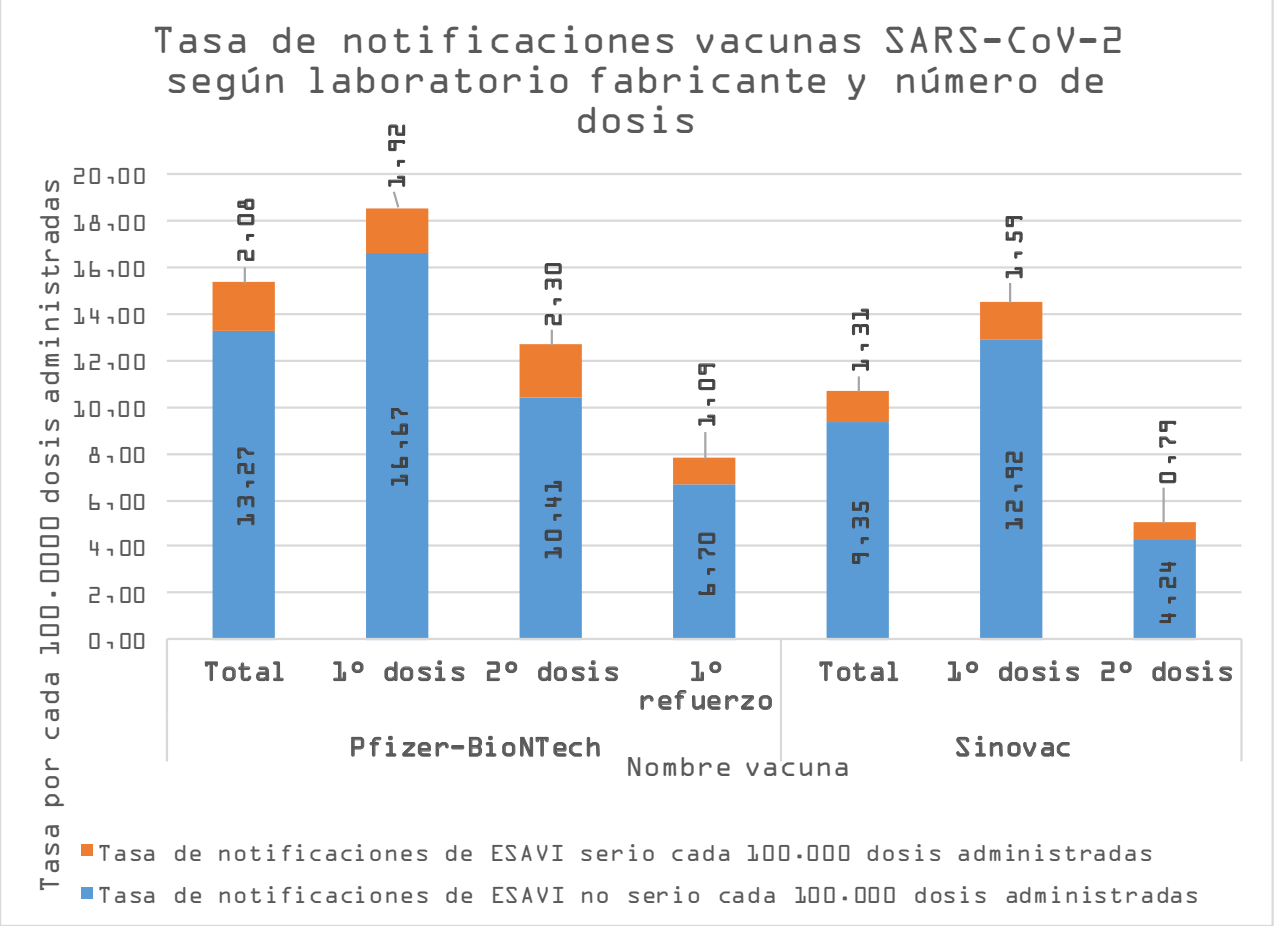


Figura 1.Tasa de notificaciones por 100.000 dosis administradas, según laboratorio fabricante y número de dosis administrada, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años.

Nota: Se excluyen las notificaciones que no indicaron el número de dosis administrada ni el laboratorio fabricante de la vacuna.

Distribución de notificaciones según sexo y grupo etario, por laboratorio fabricante

En la figura 2, se observan las tasas de notificación según sexo y grupo etario, por laboratorio fabricante. La mayor tasa de notificación para la vacuna SARS-CoV-2 Pfizer-BioNTech, se presentó en el grupo etario comprendido entre los 12-17 años, tanto para el sexo femenino como masculino, con tasas de 15,46 y 14,57 por cada 100.000 dosis administradas, respectivamente. Es importante mencionar que, si bien se han recibido un total de 4 notificaciones en el rango etario comprendido entre 05-11 años para la vacuna SARS-CoV-2 Pfizer-BioNTech, no se muestran en este gráfico debido a que el número de dosis administradas asciende a solo 864, por lo que la tasa obtenida no es representativa y no puede ser comparada con la de la vacuna SARS-CoV-2 Sinovac.

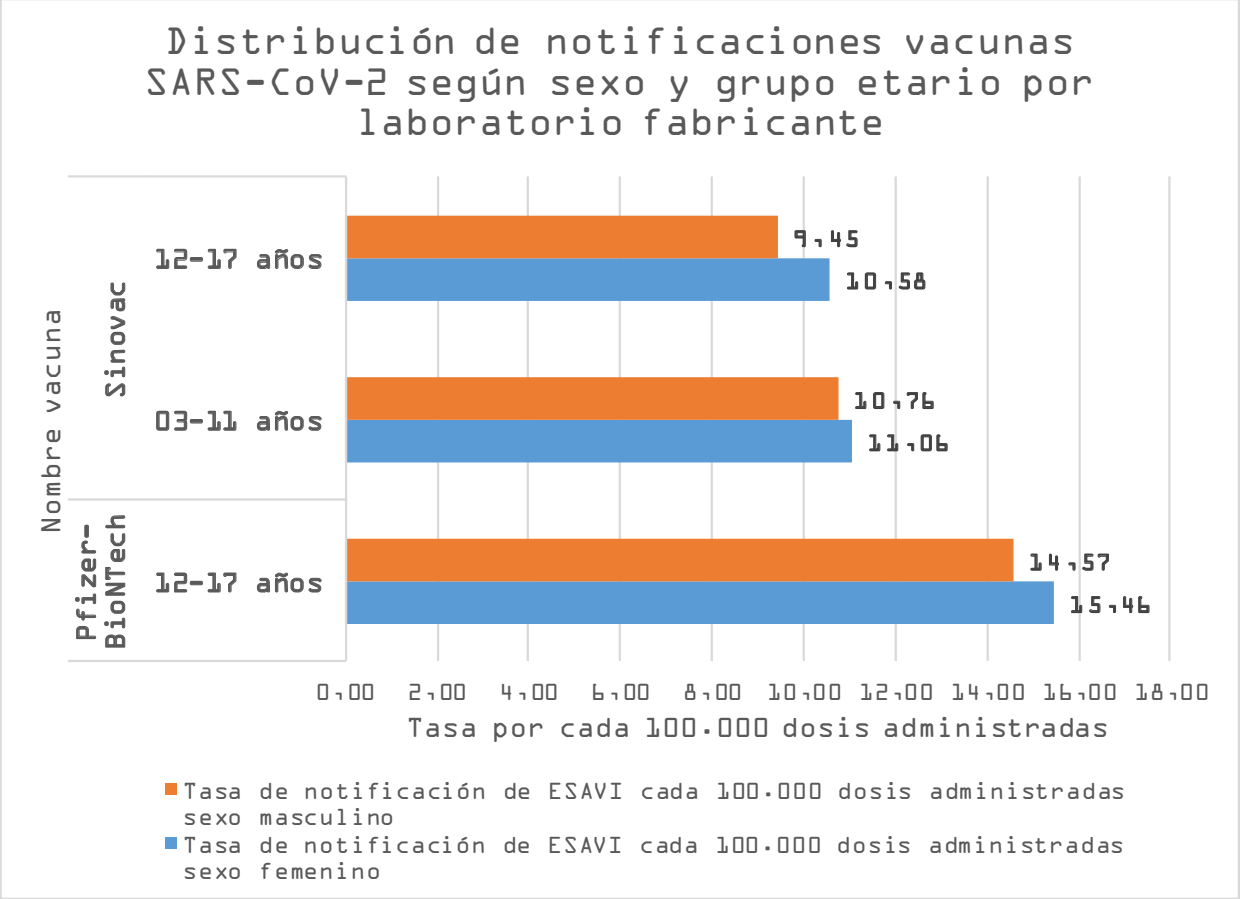


Figura 2. Distribución de notificaciones vacunas SARS CoV-2 según sexo y grupo etario, por laboratorio fabricante, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

Vacuna SARS-CoV-2 Pfizer-BioNTech

Hasta el día 26 de febrero, se habían administrado 2.071.994 dosis de la vacuna SARS-CoV-2 Pfizer-BioNTech en menores de 18 años. El total de notificaciones recibidas en el SDFV asociadas a esta vacuna fue 318, lo que corresponde a 0,02% del total de las dosis administradas y a una tasa de notificación de 15,35 reportes por cada 100.000 dosis administradas. En la Tabla 5, se desglosa el detalle por seriedad de las notificaciones recibidas.

Tabla 5. Distribución de notificaciones para la vacuna SARS-CoV-2 Pfizer-BioNTech, por seriedad, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

Seriedad	Vacuna SARS-CoV-2	N° de notificaciones	%	Tasa ESAVI por cada 100.000 dosis administradas
No	Pfizer-BioNTech	275	86,48	13,27
SI	Pfizer-BioNTech	43	13,52	2,08
Total	Pfizer-BioNTech	318	100,00	15,36

Distribución de ESAVI reportados para la vacuna SARS-CoV-2 Pfizer-BioNTech, según sexo

En la figura 3, se observan las tasas de notificación según sexo para la vacuna SARS-CoV-2 Pfizer-BioNTech, en el grupo etario de 12 a 17 años. Para el sexo femenino se recibieron un total de 161 notificaciones, lo que corresponde al 51,27% de los reportes para esta vacuna, es decir, una tasa de 15,46 por cada 100.000 dosis administradas. Para el sexo masculino se recibieron un total de 150 notificaciones, lo que corresponde al 47,77% de los reportes, es decir, una tasa de 14,57 por cada 100.000 dosis administradas. Las notificaciones que no señalaron el sexo del individuo corresponden al 0,96% del total de reportes para la vacuna SARS-CoV-2 Pfizer-BioNTech.

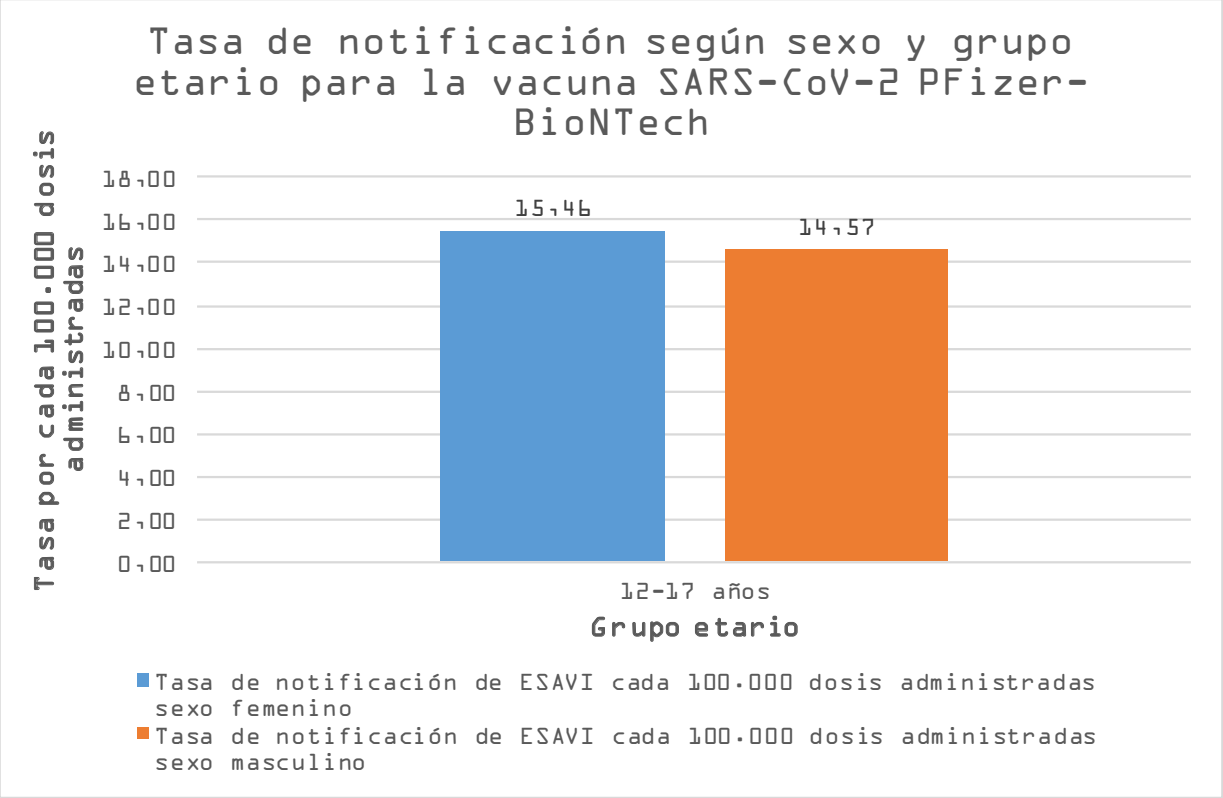


Figura 3. Tasa de notificación de ESAVI según sexo y grupo etario para la vacuna SARS-CoV-2 Pfizer-BioNTech, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

Nota aclaratoria figura 3: Es importante mencionar que se ha recibido un total de 4 notificaciones en el rango etario comprendido entre 05-11 años para la vacuna SARS-CoV-2 Pfizer-BioNTech, y el número de dosis administradas corresponde a 864, por lo que las tasas calculadas no resultan representativas, por ende, no pueden ser comparadas con las demás tasas para la misma vacuna.

Manifestaciones más frecuentes de los ESAVI No serios reportados para la vacuna SARS-CoV-2 Pfizer-BioNTech

Las manifestaciones de los eventos clasificados como no serios más frecuentemente notificados luego de la administración de la vacuna SARS-CoV-2 Pfizer-BioNTech, se resumen en la figura 4. Los eventos relacionados con reacciones en la zona de inyección presentan la mayor tasa de notificación, con una tasa de 6,37 por cada 100.000 dosis administradas, le siguen cefalea y fiebre, con una tasa de 4,39 y 2,17 por cada 100.000 dosis administradas, respectivamente. La cefalea, fatiga, mialgia, y reacciones producidas en la zona de inyección, se encuentran descritos en los ensayos clínicos realizados para esta vacuna en la población desde los 5 años.

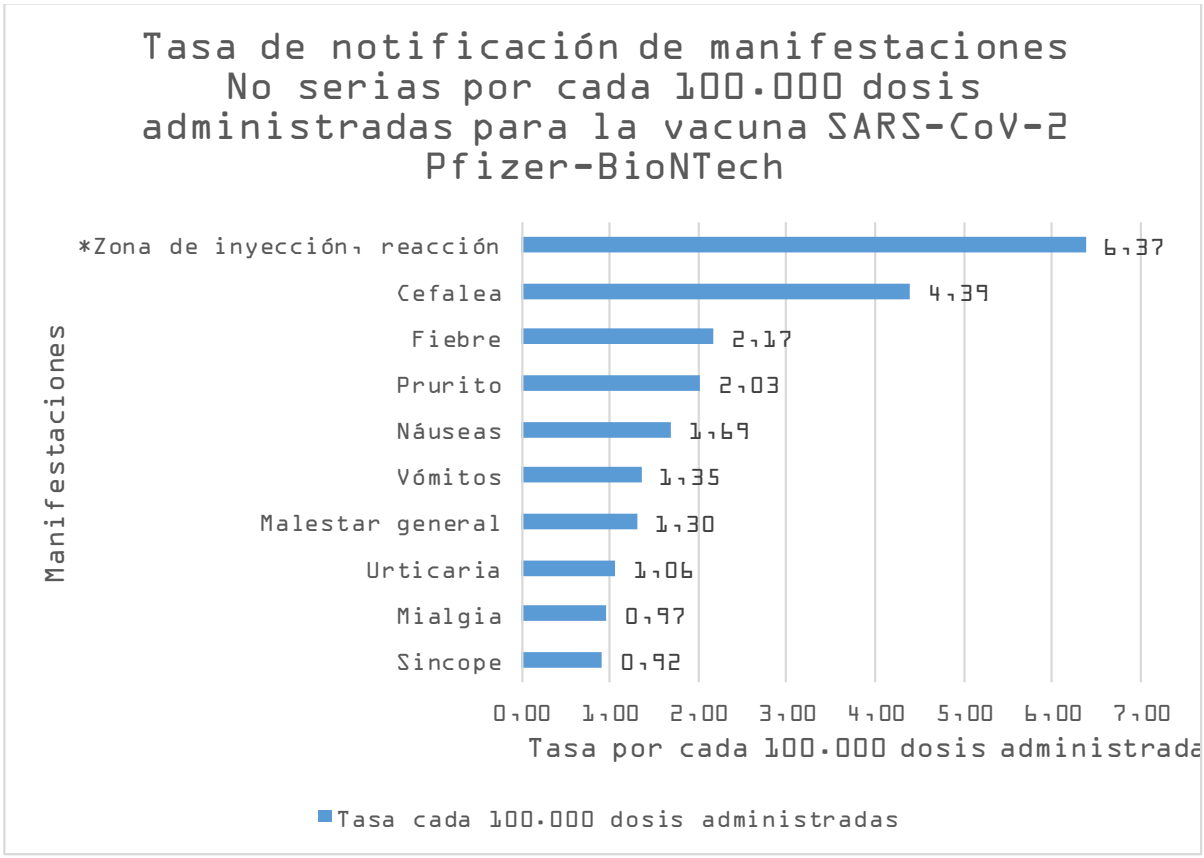


Figura 4. Tasa de notificación de manifestaciones clínicas No serias, más frecuentemente reportadas para la vacuna SARS-CoV-2 Pfizer-BioNTech, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

**zona de inyección, reacción considera: zona de inyección, dolor; zona de inyección, eritema; zona de inyección, hinchazón; zona de inyección, inflamación; zona de inyección, calentamiento; zona de inyección, sangrado; zona de inyección, endurecimiento; zona de inyección, prurito; zona de inyección, absceso*

Manifestaciones más frecuentes de los ESAVI Serios reportados para la vacuna SARS-CoV-2 Pfizer-BioNTech

En la figura 5, se observa que la manifestación seria más frecuente corresponde a miocarditis, con una tasa de 0,77 por cada 100.000 dosis administradas. Se han presentado 16 casos en el periodo evaluado, en adolescentes entre 13 a 17 años. En segundo lugar, se encuentran las manifestaciones pericarditis y reacción anafiláctica, con 0,24 notificaciones por cada 100.000 dosis administradas. Con una tasa de 0,14 por cada 100.000 dosis administradas, en tercer lugar, se encuentra la manifestación convulsiones.

Es importante señalar que, tanto la miocarditis como la pericarditis, son eventos que han sido evaluados como una señal de seguridad. El Comité de Evaluación de Riesgos en Farmacovigilancia de la Unión Europea (PRAC, por sus siglas en inglés), encargado de evaluar todos los aspectos de la gestión de riesgos de los medicamentos de uso humano en ese continente, concluyó, tras la evaluación detallada de los datos disponibles, que existe una relación causal plausible entre los casos reportados y la administración de las vacunas ARNm. Esto quiere decir, que es razonable suponer que la vacuna puede haber sido un elemento causal de la afección. No obstante, la frecuencia de aparición de casos de miocarditis y/o pericarditis luego de la administración de estas vacunas sigue siendo muy rara, detectándose tasas de 0,26 por 10.000 (2,6 por 100.000) casos extra de miocarditis, vale decir sobre nivel basal, en hombres de 12 a 29 años, comparados con personas no vacunadas.

También es importante mencionar que se presentaron, con una tasa de 0,05 por cada 100.000 dosis administradas, casos de cianosis, hipertonía, nefritis e insuficiencia cardiaca. Estos no se informaron en el gráfico debido a que no se ubican dentro de las 10 primeras manifestaciones más frecuentes.

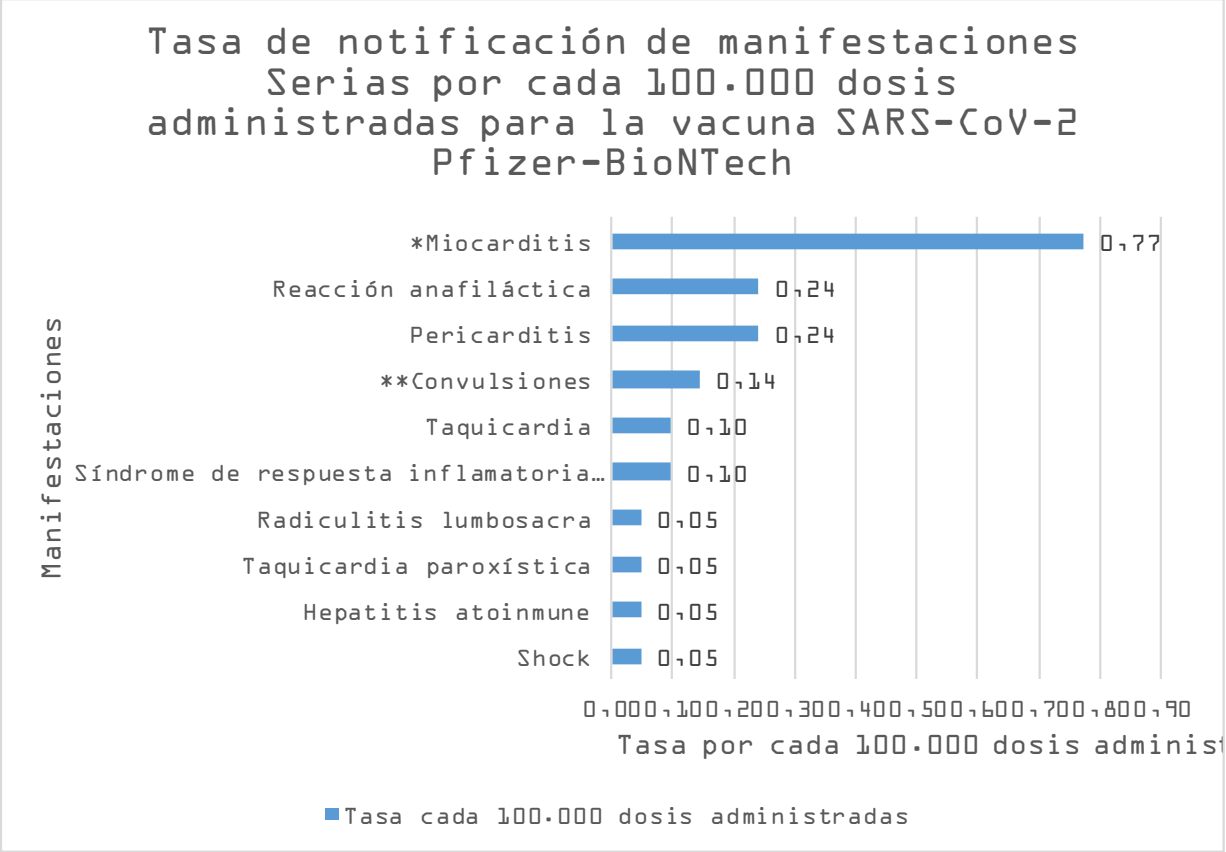


Figura 5. Tasa de notificación de manifestaciones clínicas serias más frecuentemente reportadas, para la vacuna SARS-CoV-2 Pfizer-BioNTech periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

**miocarditis considera: miocarditis y miopericarditis. ** convulsiones considera: convulsiones, convulsiones focales y convulsiones tónico/clónicas*

Eventos de especial interés reportados para la vacuna SARS-CoV-2 Pfizer-BioNTech

Los eventos de especial interés (AESI, por sus siglas en inglés – eventos de importancia médica predefinidos que necesitan ser monitoreados y confirmados por estudios específicos) notificados con posterioridad a la administración de la vacuna SARS-CoV-2 Pfizer-BioNTech, se resumen en la figura 6. Esta clasificación es independiente de si la notificación fue catalogada como seria o no seria, por lo que puede repetirse con las manifestaciones señaladas en las secciones anteriores de este informe. Se observa que el evento miocarditis fue el que presentó la mayor tasa de notificación, alcanzando 0,87 por cada 100.000 dosis administradas; le sigue, en segundo lugar, convulsiones, con 0,58 por cada 100.000 dosis administradas y, en tercer lugar, reacción anafiláctica y pericarditis, las que se presentaron con una tasa de 0,29 por cada 100.000 dosis administradas. Todas las tasas expresadas como 0,00 en la figura 6, son AESI que no se presentaron en las notificaciones recibidas para esta vacuna.

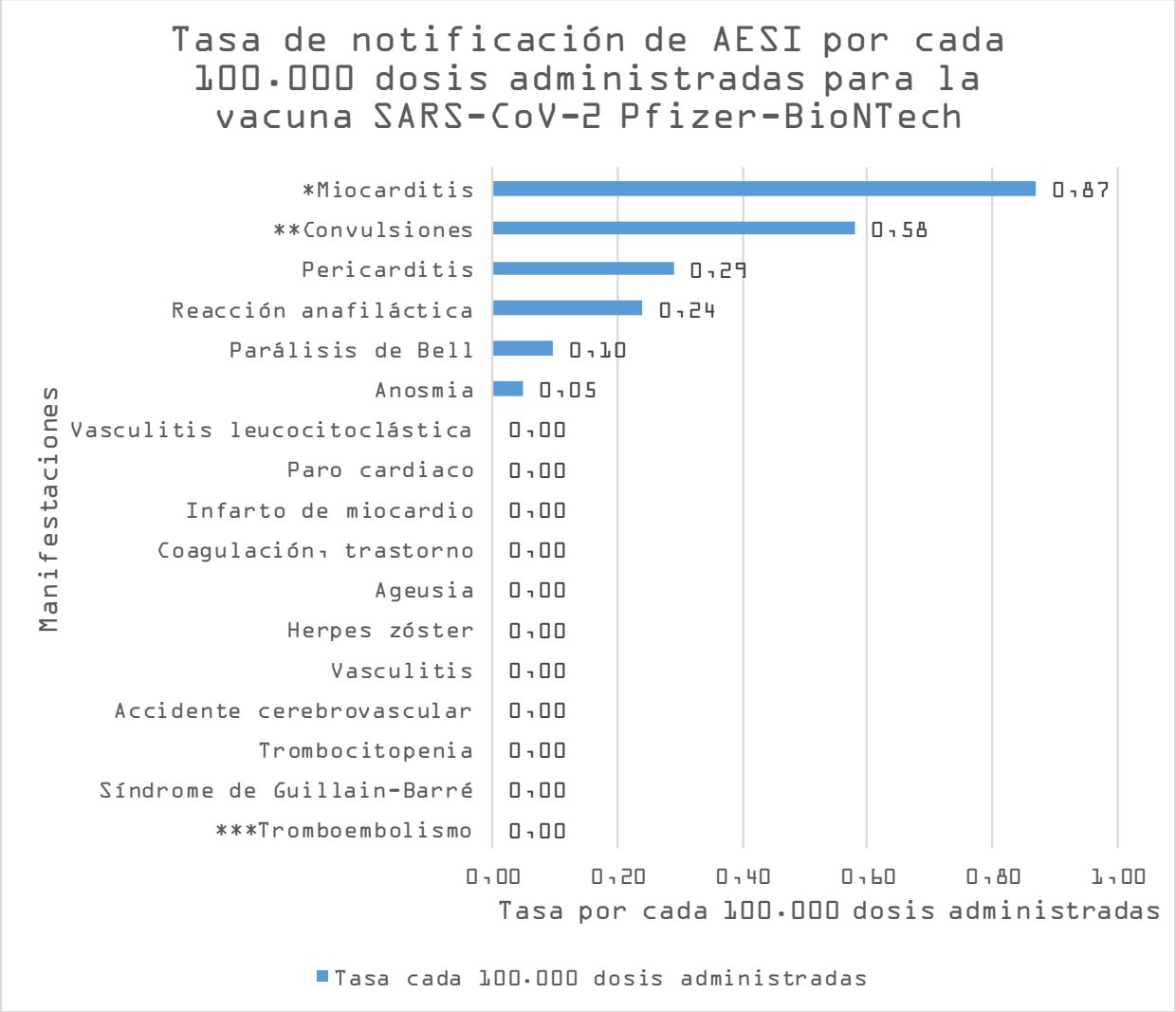


Figura 6. Tasa de notificación de manifestaciones clínicas de especial interés reportadas para la vacuna SARS-CoV-2 Pfizer-BioNTech, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

**miocarditis considera: miocarditis y miopericarditis. **convulsiones considera: convulsiones, convulsiones focales y convulsiones tónico/clónicas. ***tromboembolismo considera: trombosis, tromboembolismo y embolismo.*

Comparación de ESAVI presentados por la vacuna SARS-CoV-2 Pfizer-BioNTech según número de dosis

ESAVI no serios según número de dosis vacuna SARS-CoV-2 Pfizer-BioNTech

La comparación de las manifestaciones no serias más frecuentemente notificadas según el número de dosis administradas, de vacuna SARS-CoV-2 Pfizer-BioNTech, se presentan en la tabla 6. En concordancia con lo descrito anteriormente, las manifestaciones más frecuentes corresponden a reacciones locales, con una tasa de 8,59 por cada 100.000 primeras dosis administradas, mientras

que, al administrarse la segunda dosis, esta tasa desciende a 3,92 por cada 100.000 segundas dosis administradas y en la dosis de 1° refuerzo se presenta con 4,71 por cada 100.000 dosis administradas. La cefalea se presenta como la segunda manifestación más frecuente, con tasas similares tanto para la primera como para la segunda dosis, de 4,36 y 4,46 por cada 100.000 dosis administradas, respectivamente, y en el 1° refuerzo se presentó con una tasa de 2,53 por cada 100.000 dosis administradas. La tercera manifestación más frecuente corresponde a prurito, con una tasa de 2,82 por cada 100.000 primeras dosis administradas, pero, si observamos la misma manifestación con la segunda dosis, la tasa disminuye a 1,62 por cada 100.000 dosis administradas; en cuanto a la tasa presentada con su uso como 1° refuerzo, esta disminuye a 0,72 por cada 100.000 dosis administradas.

A nivel general, los datos muestran que todas las manifestaciones presentadas más frecuentemente con la primera dosis, disminuyeron con la segunda dosis y con la dosis de 1° refuerzo.

Tabla 6. Comparación de las tasas de notificación de los ESAVI No serios más frecuentes, de acuerdo al número de dosis para la vacuna SARS-Cov-2 Pfizer-BioNTech, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

Manifestaciones	Tasa de notificación de ESAVI No serio cada 100.000 dosis administradas para la vacuna SARS-CoV-2 Pfizer-BioNTech		
	1° dosis	2° dosis	1° refuerzo
Zona de inyección, reacción*	8,59	3,92	4,71
Cefalea	4,36	4,46	2,53
Prurito	2,82	1,62	0,72
Sincope	1,92	0,27	0,36
Náuseas	1,92	1,62	0,90
Fiebre	1,54	3,11	1,27
Malestar general	1,54	1,08	0,90
Urticaria	1,41	0,68	0,36
Vómitos	1,41	1,22	0,72
Mareo	1,03	0,81	0,36

**zona de inyección, dolor; zona de inyección, eritema; zona de inyección, hinchazón; zona de inyección, calentamiento; zona de inyección, inflamación; zona de inyección, endurecimiento; zona de inyección, sangrado; zona de inyección, prurito; zona de inyección, absceso.*

Nota aclaratoria tabla 6: se han recibido en el SDFV, 3 notificaciones no serias para esta vacuna administrada como 2° refuerzo, administrándose, a la fecha de corte de este informe, 151 dosis de 2° refuerzo, por lo que las tasas calculadas no resultan representativas y por ende no pueden ser comparadas con las del esquema primario de vacunación, por ello no se incluyen en la tabla anterior.

ESAVI Serios, según número de dosis, vacuna SARS-CoV-2 Pfizer-BioNTech

La comparación de las manifestaciones serias notificadas según el número de dosis administradas de la vacuna SARS-CoV-2 Pfizer-BioNTech, se presentan en la tabla 7. Las manifestaciones más frecuentemente notificadas con la primera dosis de la vacuna SARS-CoV-2 Pfizer-BioNTech son; reacción anafiláctica en primer lugar, con una tasa de 0,64 por cada 100.000 primeras dosis administradas; en segundo lugar, se encuentra síndrome de respuesta inflamatoria multisistémica, con 0,26 por cada 100.000 primeras dosis administradas. Se presentaron 2 casos de este síndrome, los cuales fueron evaluados por el Comité de Farmacovigilancia de Vacunas como inconsistentes con el proceso de vacunación, debido a que estuvieron en contacto con pacientes con COVID-19, previo a la vacunación, y esa puede ser una razón más plausible. En tercer lugar, se encuentran, con una tasa de 0,13 por 100.000 primeras dosis administradas, las manifestaciones miocarditis, convulsiones, pericarditis, encefalitis, hepatitis autoinmune, insuficiencia cardiaca, hipertonía y cianosis. Para la segunda dosis, destaca, en primer lugar, la miocarditis, con una tasa de 1,62 por cada 100.000 segundas dosis administradas; en segundo lugar se encuentra pericarditis, con una

tasa de 0,41 por cada 100.000 segundas dosis administradas, mientras que, en tercer lugar, se encuentran reacción anafiláctica y convulsiones, con una tasa de 0,14 por cada 100.000 segundas dosis administradas, cada una. Para el 1° refuerzo, con una tasa de 0,36 por cada 100.000 dosis administradas se observa miocarditis en primer lugar, mientras que, con una tasa de 0,18 por cada 100.000 dosis administradas, en segundo lugar se encuentran convulsiones y pericarditis. Los hallazgos de reacciones cardiológicas se condicen con lo detectado a nivel internacional, tal como se señaló en párrafos anteriores.

Para todos los eventos serios descritos, el ISP ha realizado seguimiento de su evolución clínica y recopilación de antecedentes, con el objetivo de contar con toda la información necesaria que permita evaluar los casos y detectar si existieron antecedentes previos, así como factores de riesgo y/o causas alternativas que pudieran influir en la aparición del evento observado.

Tabla 7. Comparación de las tasas de notificación de los ESAVI Serios más frecuentes, de acuerdo al número de dosis para la vacuna SARS-Cov-2 Pfizer-BioNTech, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

Manifestaciones	Tasa de notificación de ESAVI Serio cada 100.000 dosis administradas para la vacuna SARS-CoV-2 Pfizer-BioNTech		
	1°dosis	2° dosis	1°refuerzo
Reacción anafiláctica	0,64	0,14	0,00
Síndrome de respuesta inflamatoria sistémica	0,26	0,00	0,00
Convulsiones*	0,13	0,14	0,18
Miocarditis**	0,13	1,62	0,36
Pericarditis	0,13	0,41	0,18
Encefalitis	0,13	0,00	0,00
Hepatitis autoinmune	0,13	0,00	0,00
Insuficiencia cardiaca	0,13	0,00	0,00
Hipertonía	0,13	0,00	0,00
Cianosis	0,13	0,00	0,00

**convulsiones considera: convulsiones, convulsiones focales y convulsiones tónico/clónicas. **miocarditis considera; miocarditis y miopericarditis.*

Vacuna SARS-CoV-2 Sinovac

Hasta el día 26 de febrero, se habían administrado 4.874.599 dosis de la vacuna SARS-CoV-2 Sinovac a menores de 18 años. El total de notificaciones recibidas en el SDFV asociadas a esta vacuna fue de 520, lo que corresponde a 0,01% del total de dosis administradas y a una tasa de notificación de 10,67 reportes cada 100.000 dosis. En la Tabla 8, se desglosa el detalle por seriedad de las notificaciones recibidas.

Tabla 8. Distribución de notificaciones para la vacuna SARS-CoV-2 Sinovac, por seriedad, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

Seriedad	Vacuna SARS-CoV-2	N° de notificaciones	%	Tasa ESAVI cada 100.000 dosis administradas
No	Sinovac	456	87,69	9,35
SI	Sinovac	64	12,31	1,31
Total	Sinovac	520	100,00	10,67

Distribución según sexo y grupo etario de ESAVI vacuna SARS-CoV-2 Sinovac

En la figura 7, se pueden observar las tasas de notificación según grupo etario y sexo para la vacuna SARS-CoV-2 Sinovac. Para ello, se establecieron dos grupos, el primero corresponde al tramo 3-11 años y el segundo 12-17 años. Al compararlos, se observa una mayor tasa de notificación para el grupo entre los 3 y 11 años de edad, dentro del cual se contabilizó un total de 191 notificaciones de pacientes de sexo femenino, lo que corresponde al 36,73% de los reportes para esta vacuna, es decir, una tasa de 11,06 por cada 100.000 dosis administradas. Para el sexo masculino se recibieron un total de 188 notificaciones, lo que corresponde al 36,15% de los reportes para esta vacuna, es decir, una tasa de 10,76 por cada 100.000 dosis administradas. El segundo grupo etario evaluado en este informe corresponde al tramo 12-17 años. Para el sexo femenino se recibieron un total de 73 notificaciones, lo que corresponde al 14,04% de los reportes para esta vacuna, es decir, una tasa de 10,58 por cada 100.000 dosis administradas, mientras que para el sexo masculino se recibieron un total de 67 notificaciones, lo que corresponde al 12,88% de los reportes, es decir, una tasa de 9,45 por cada 100.000 dosis administradas. Cabe mencionar que 1 reporte no declaró el sexo del paciente, lo que corresponde al 0,20% de los reportes.

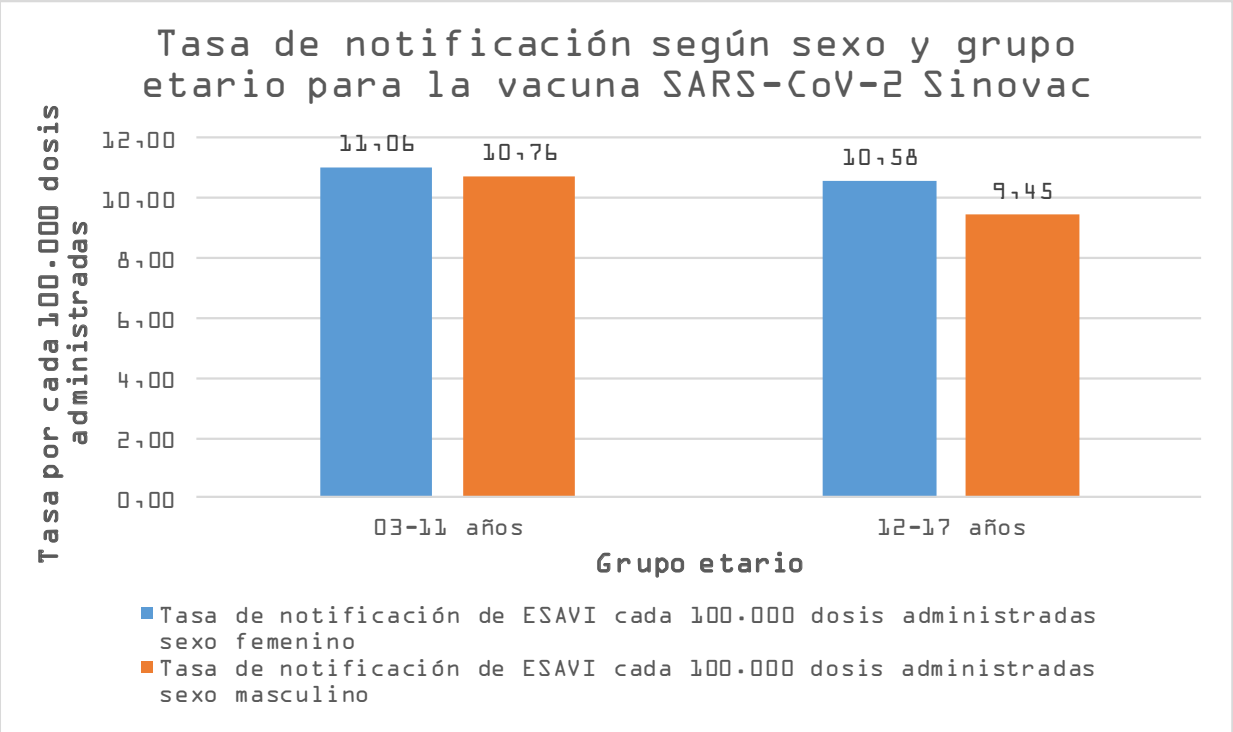


Figura 7. Tasa de notificación de ESAVI según sexo y grupo etario para la vacuna SARS-CoV-2 Sinovac, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

Manifestaciones más frecuentes de los ESAVI No serios, reportados para la vacuna SARS-CoV-2 Sinovac

Las manifestaciones no serias más frecuentemente notificadas posteriormente a la administración de la vacuna SARS-CoV-2 Sinovac, se resumen en la figura 8, en la cual se muestra que la más frecuente corresponde a las reacciones locales presentadas en la zona de inyección, con una tasa de 3,26 por cada 100.000 dosis administradas. Le siguen prurito y cefalea, con tasas de 1,99 y de 1,60 por cada 100.000 dosis administradas, respectivamente. Las manifestaciones: cefalea, fiebre, mialgia, náuseas, malestar general y reacciones producidas en la zona de inyección, se encuentran descritas en los ensayos clínicos realizados para esta vacuna en la población desde los 3 años de edad.

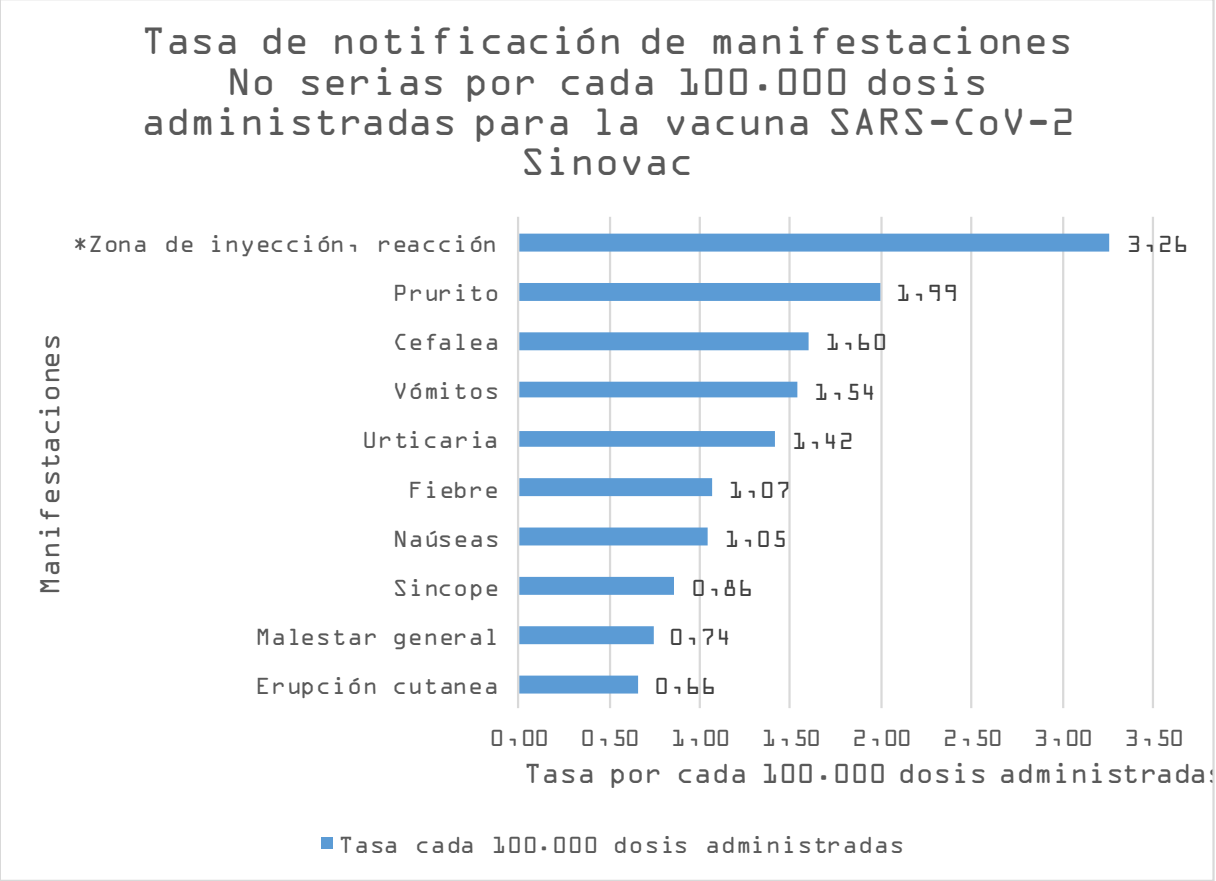


Figura 8. Tasa de notificación de manifestaciones clínicas no serias más frecuentemente reportadas para la vacuna SARS-CoV-2 Sinovac, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

*zona de inyección reacción considera: zona de inyección, dolor; zona de inyección, eritema; zona de inyección, hinchazón.

Manifestaciones más frecuentes de los ESAVI Serios, reportados para la vacuna SARS-CoV-2 Sinovac

Los eventos clasificados como serios, más frecuentemente notificados luego de la administración de la vacuna SARS-CoV-2 Sinovac, se resumen en la figura 9. Se puede observar que la manifestación seria más frecuente es convulsiones, la que se presentó con una tasa de 0,29 por cada 100.000 dosis administradas. El segundo lugar corresponde a reacción anafiláctica, con una tasa de 0,27 por cada 100.000 dosis administradas; en el tercero se encuentran Síndrome de Guillain-Barré y tromboembolismo con 0,06 notificaciones por cada 100.000 dosis administradas. Es importante mencionar que también se presentó, con una tasa de 0,02 por cada 100.000 dosis administradas, bloqueo aurículo ventricular, cuadriparesia, hepatitis fulminante, miocarditis, pericarditis,

encefalomielitis, enfermedad de kawasaki. Éstos no se informaron en el gráfico, debido a que no se presentaron dentro de las 10 primeras manifestaciones más frecuentes.

Para todos los eventos serios descritos, el ISP ha realizado seguimiento de su evolución clínica y recopilación de antecedentes, con el objetivo de contar con toda la información necesaria que permita evaluar los casos y detectar si existieron antecedentes previos, factores de riesgo y/o causas alternativas que pudieran influir en la aparición del evento observado.

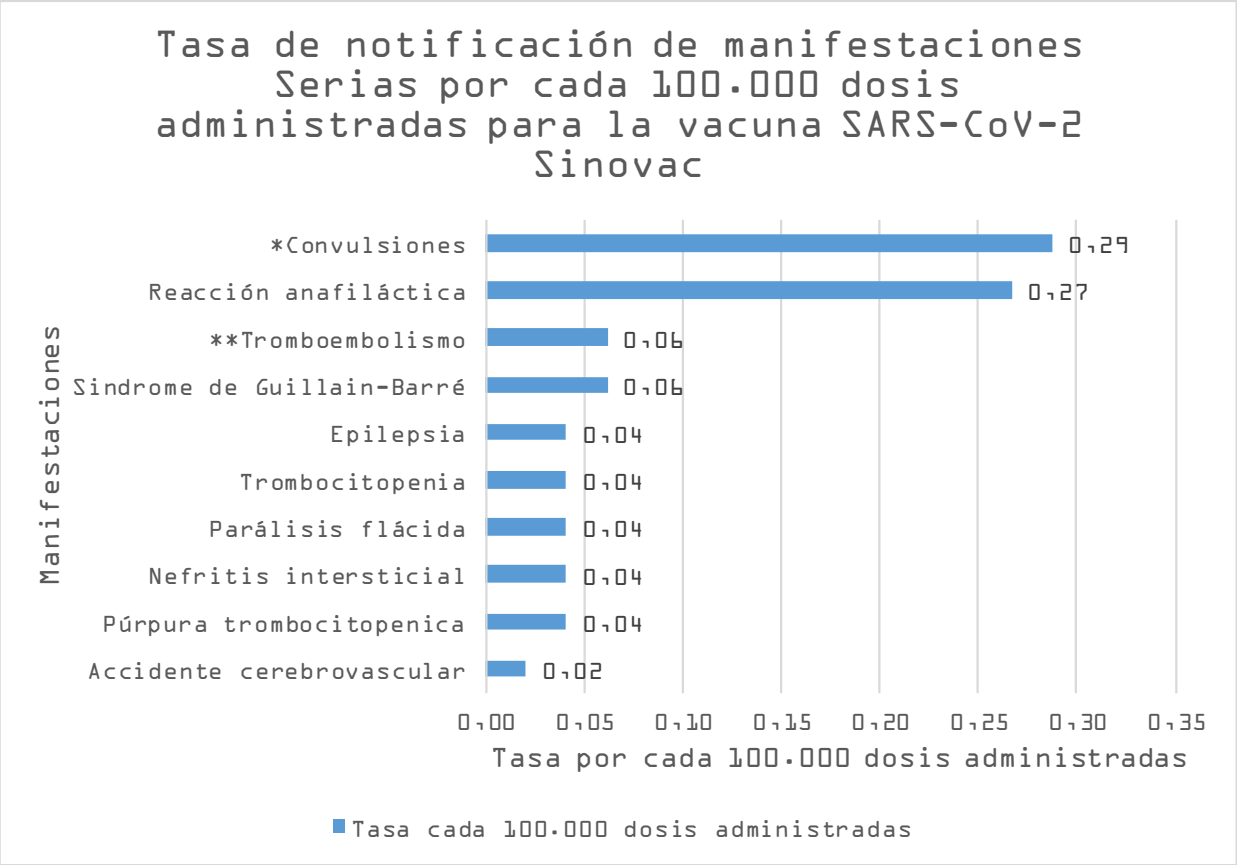


Figura 9. Tasa de notificación de manifestaciones clínicas serias más frecuentemente reportadas, para la vacuna SARS-CoV-2 Sinovac periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

**convulsiones considera: convulsiones, convulsiones focales y convulsiones tónico/clónicas, **tromboembolismo considera: trombosis, tromboembolismo y embolismo*

Eventos de especial interés reportados para la vacuna SARS-CoV-2 Sinovac

Los eventos de especial interés notificados luego de la administración de la vacuna SARS-CoV-2 Sinovac se resumen en la figura 10. Esta clasificación es independiente de si la notificación fue catalogada como seria o no seria, por lo que puede repetirse con las manifestaciones señaladas en

las secciones anteriores de este informe. Se observa que convulsiones fue el AESI que presentó una mayor tasa, con 0,70 notificaciones por cada 100.000 dosis administradas. El segundo evento más frecuente corresponde a reacción anafiláctica, con una tasa de 0,27 por cada 100.000 dosis administradas. En tercer lugar, se encuentra Síndrome de Guillain-Barré y tromboembolismo, con una tasa de 0,06 por cada 100.000 dosis administradas. Todas las tasas expresadas como 0,00 en la figura 10, son AESI que no se presentaron en las notificaciones recibidas para esta vacuna.

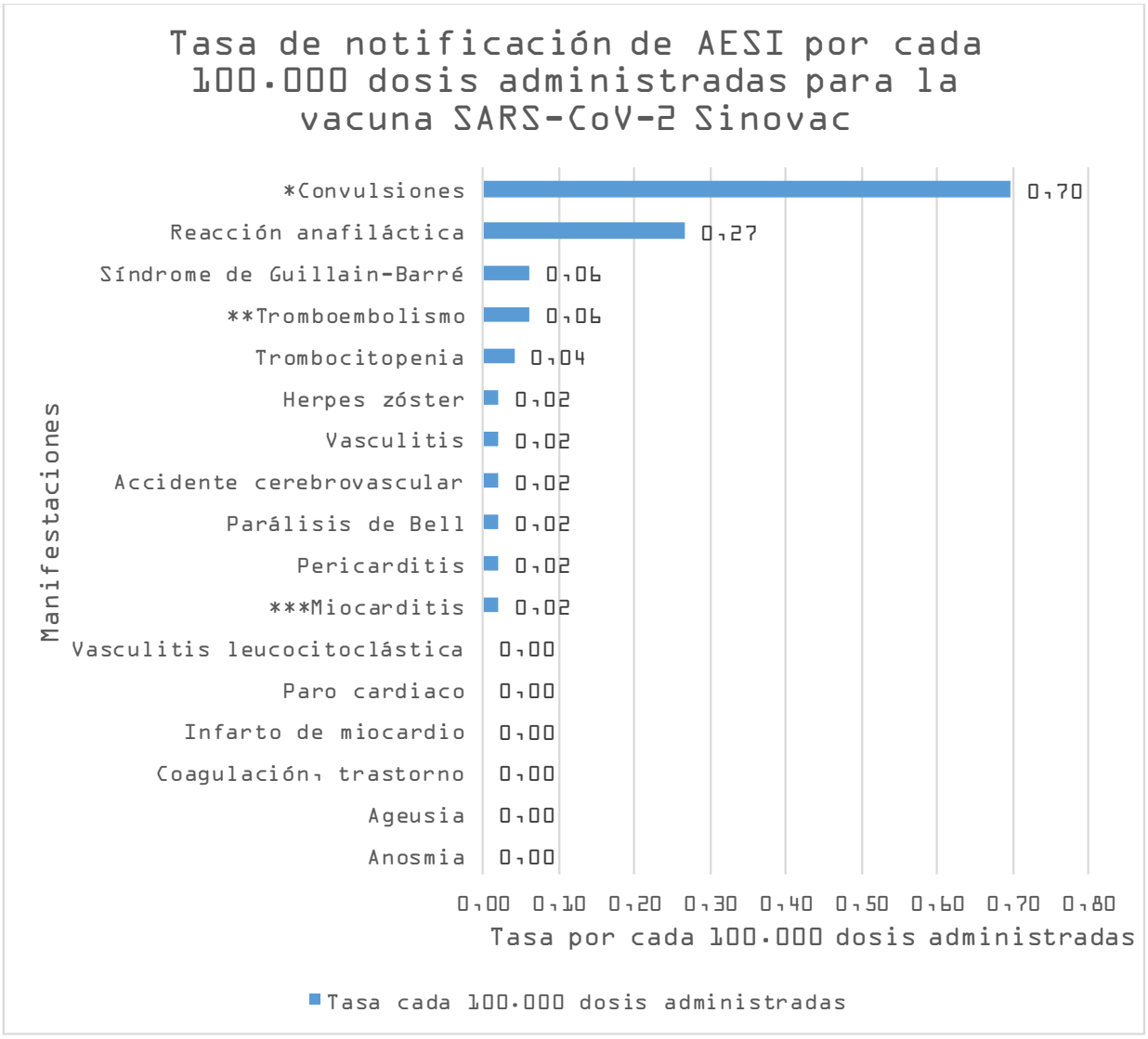


Figura 10. Tasa de notificación de manifestaciones clínicas de especial interés, reportadas para la vacuna SARS-CoV-2 Sinovac periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

**convulsiones considera: convulsiones, convulsiones focales y convulsiones tónico/clónicas. **tromboembolismo considera: trombosis, Tromboembolismo y embolismo. ***miocarditis considera: miocarditis y miopericarditis*

Comparación de ESAVI presentados por la vacuna SARS-CoV-2 Sinovac, según número de dosis

ESAVI no serios según número de dosis vacuna SARS-CoV-2 Sinovac

La comparación de las manifestaciones no serias más frecuentemente notificadas según el número de dosis administradas de la vacuna SARS-CoV-2 Sinovac, se presentan en la tabla 9. Se observa que la manifestación más frecuente corresponde a reacciones en la zona de inyección, tanto para la primera como para la segunda dosis, con tasas de 4,56 y 1,70 por cada 100.000 dosis administradas, respectivamente. En relación a la primera dosis, la segunda manifestación más frecuente corresponde a prurito, con una tasa de 2,94 por cada 100.000 dosis administradas y, en tercer lugar, se observó urticaria, con una tasa de 2,05 por cada 100.000 dosis administradas. Por otro lado, si se observan las manifestaciones producidas de forma posterior a la administración de la segunda dosis, la segunda manifestación más frecuente corresponde a cefalea, con una tasa de 0,96 por cada 100.000 dosis administradas y, en tercer lugar, se encuentra vómitos, con una tasa de 0,87 por cada 100.000 dosis administradas. Al comparar ambas dosis se evidencia que, en general, se presentan menores tasas de ESAVI no serios con la 2° dosis. Es importante mencionar que para el 1° refuerzo solo se recibieron 2 notificaciones en los que se reportó, eritema, prurito, y vejiga neurógena. Sumado a lo anterior, las dosis administradas para esta vacuna corresponden a 207, por lo que el valor de la tasa no resulta representativo para realizar una comparación entre dosis de la misma vacuna.

Tabla 9. Comparación de las tasas de notificación de los ESAVI No serios más frecuentes, de acuerdo al número de dosis para la vacuna SARS-Cov-2 Sinovac, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

Manifestaciones	Tasa de notificación de ESAVI No serio cada 100.000 dosis administradas para la vacuna SARS-CoV-2 Sinovac		
	1° dosis	2° dosis	1°refuerzo
Zona de inyección, reacción*	4,56	1,70	0,00
Prurito	2,94	0,79	0,00
Urticaria	2,05	0,61	0,00
Cefalea	1,93	0,96	0,00
Vómitos	1,90	0,87	0,00
Náuseas	1,39	0,48	0,00
Fiebre	1,39	0,61	0,00
Sincope	1,28	0,39	0,00
Erupción cutánea	0,97	0,26	0,00
Malestar general	0,93	0,48	0,00

*zona de inyección reacción considera: zona de inyección, dolor; zona de inyección, eritema; zona de inyección, hinchazón.

ESAVI serios según número de dosis vacuna SARS-CoV-2 Sinovac.

La comparación de las manifestaciones serias más frecuentemente notificadas según el número de dosis administradas de la vacuna SARS-CoV-2 Sinovac se presentan en la tabla 10. La manifestación más frecuente que se presentó posteriormente a la primera dosis, fue convulsiones, con una tasa de 0,43 por cada 100.000 dosis administradas. En segundo lugar, se encuentra reacción anafiláctica, con una tasa de 0,39 por 100.000 dosis administradas. La tercera, corresponde a trombocitopenia y epilepsia, con una tasa de 0,08 por cada 100.000 dosis administradas. En relación a la segunda dosis, en primer lugar, se encuentran reacción anafiláctica y Síndrome de Guillain-Barré, con tasas de 0,13 por cada 100.000 dosis administradas. En segundo lugar, se encuentra convulsiones, con una tasa de 0,09 por cada 100.000 dosis administradas. Es importante mencionar que para el 1° refuerzo no se recibieron notificaciones de ESAVI clasificadas como serias.

Tabla 10. Comparación de las tasas de notificación de los ESAVI Serios más frecuentes, de acuerdo al número de dosis del esquema primario para la vacuna SARS-Cov-2 Sinovac, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

Manifestaciones	Tasa de notificación de ESAVI serio cada 100.000 dosis administradas para la vacuna SARS-CoV-2 Sinovac		
	1ra dosis	2da dosis	1° refuerzo
Convulsiones*	0,43	0,09	0,00
Reacción anafiláctica	0,39	0,13	0,00
Trombocitopenia	0,08	0,00	0,00
Epilepsia	0,08	0,00	0,00
Encefalomielitis	0,04	0,00	0,00
Encefalitis	0,04	0,00	0,00
Tromboembolismo**	0,04	0,00	0,00
Accidente cerebrovascular	0,04	0,00	0,00
Síndrome de respuesta inflamatoria multisistémica	0,04	0,00	0,00
Síndrome de Guillain-Barré	0,04	0,13	0,00

**convulsiones considera: convulsiones, convulsiones focales y convulsiones tónico/clónicas. **tromboembolismo considera: trombosis, tromboembolismo y embolismo*

Consideraciones de este informe

- Este es un informe acumulativo, que se actualiza en función de los nuevos reportes recibidos, abarcando, de esta forma, el periodo comprendido desde el inicio de la campaña de vacunación en adolescentes (01 de marzo 2020) hasta el 26 de febrero 2022.
- Las dosis administradas incluyen el periodo del 24 de diciembre de 2020 al 26 de febrero de 2022 en personas entre 03-17 años.
- La información presentada en este informe se basa en las notificaciones que fueron reportadas al SDFV de manera espontánea, y no sobre el número total de personas que experimentan un evento adverso en el país, ya que se asume que no se informan todos los eventos ocurridos en la población, dado los sesgos propios del sistema de vigilancia pasiva.
- Los eventos notificados como errores programáticos debido a la administración de estas vacunas en población pediátrica antes de ser autorizado su uso en ese grupo etario, no fueron considerados en el presente informe. En el periodo de tiempo que abarca este informe, se reportaron 15 notificaciones de este tipo, todas calificadas como NO serias, que representan el 1,73% de las notificaciones recibidas para menores de 18 años.
- Los Eventos Adversos de Interés Especial o Adverse Event of Special Interest (AESI), corresponden a un evento médicamente significativo, definido e identificado recientemente, que tiene el potencial de tener una asociación causal con una vacuna, pero que aún no se confirma. Este tipo de evento debe ser monitorizado cuidadosamente y confirmado por estudios específicos adicionales. Ejemplo de AESI: Síndrome de Trombosis con Trombocitopenia, Síndrome de Guillain Barré, Miocarditis/Pericarditis, Anafilaxias, entre otros

Conclusiones

- Durante el periodo estudiado (01 de marzo 2021 hasta el 26 de febrero 2022), fueron administradas 6.946.593 dosis de vacunas SARS-CoV-2 a menores de 18 años y se **reportaron 868 ESAVI**, correspondientes al 0,01% de las dosis administradas, vale decir, se presentó una tasa de 12,50 notificaciones de ESAVI por 100.000 dosis administradas.
- Las manifestaciones clínicas No serias más frecuentemente reportadas para estas vacunas se encuentran descritas entre los eventos adversos que ya habían sido observados en los ensayos clínicos realizados.
- Los ESAVI clasificados como No serios corresponden al 87,67% del total de eventos reportados, vale decir, una tasa de 10,96 notificaciones de ESAVI no serios por 100.000 dosis administradas de vacunas SARS-CoV-2.
- Los ESAVI clasificados como serios corresponden al 12,33% del total de eventos reportados, vale decir, una tasa de notificaciones de 1,54 ESAVI serios por 100.000 de dosis administradas.
- En resumen:

Número de ESAVI totales	% de ESAVI en la población vacunada	Tasa de notificación x 100.000 habitantes
868	0,01 %	12,50

ESAVI clasificados como No serios	% de ESAVI en la población vacunada	Tasa de notificación x 100.000 habitantes
761	0,01 %	10,96

ESAVI clasificados como serios	% de ESAVI en la población vacunada	Tasa de notificación x 100.000 habitantes
107	0,002 %	1,54

- En la actual campaña de vacunación contra COVID-19 se han administrado un total de 4.874.599 dosis de la vacuna inactivada SARS-CoV-2 Sinovac, lo que representa un 70,17% del total de las dosis administradas en el país; no obstante, presenta una menor tasa de notificación de ESAVI en comparación a la vacuna SARS-CoV-2 Pfizer-BioNTech.

Referencias

1. Instituto de Salud Pública. Vacunas COVID-19 [Consultada: 02/05/2022].Disponible en https://www.ispch.cl/isp-covid-19/vacunas-covid-19/
2. Ministerio de Salud de Chile. Calendario de vacunación masiva covid-19. [Consultada: 11/01/2022]. Disponible https://www.minsal.cl/calendario-de-vacunacion-masiva-contra-covid-19/calendarios-anteriores-de-vacunacion-masiva-contra-covid-19/
3. Ministerio de Salud de Chile. Modifica resolución exenta N°1138 de 2020, de la subsecretaria de salud pública que aprueba lineamientos técnicos operativos vacunación SARS-CoV-2 3 nov 2021. [Consultada: 31/12/2021]. disponible en https://www.minsal.cl/wp-content/uploads/2021/12/Modifica-Res.-Exenta-N%C2%B01138-de-2020-de-la-Subsecretar%C3%ADa-de-Salud-P%C3%BAblica-Lineamientos-SARS-CoV-2-03.11.2021.pdf
4. Instituto de Salud Pública. Subdepartamento Farmacovigilancia. Base de datos RAM-ESAVI, Red-RAM, NOTI RAM ESAVI. [Consultada: 25/04/2022].
5. Ministerio de Salud. [En línea]. Estudio sobre la efectividad de dosis de refuerzo [publicado: octubre 2021. consultado: 04/11/2021] Disponible en: https://www.minsal.cl/wp-content/uploads/2021/10/2021-10-07- EFECTIVIDAD-DOSIS-DE-REFUERZO_ESP.pdf
6. Pozzetto, B., Legros, V., Djebali, S. et al. Immunogenicity and efficacy of heterologous ChAdOx1–BNT162b2 vaccination. Nature (2021). Disponible en https://doi.org/10.1038/s41586-021-04120-y
7. Instituto de Salud Pública. [En línea] Fichas informativas de vacunas COVID-19. [Consultada: 02/05/2022]. Disponible en: https://www.ispch.cl/isp-covid-19/fichas-informativas-de-vacunas-covid-19/
8. CDC. Allergic Reactions Including Anaphylaxis After Receipt of the First Dose of Moderna COVID-19 Vaccine — United States, December 21, 2020–January 10, 2021 [Consultada: 30/12/2021], Disponible en: https://www.cdc.gov/mmwr/volumes/70/wr/mm7004e1.htm
9. Ministerio de Salud de Chile, Dosis de refuerzo en la campaña de vacunación contra SARS-CoV-2 en chile fase3: niños, niñas y adolescentes. [Consultada: 10/01/2022]. Disponible en: https://www.minsal.cl/wp-content/uploads/2021/12/Dosis-de-Refuerzo-en-la-Campa%C3%B1a-de-Vacunaci%C3%B3n-contra-SARS-CoV-2-Fase-3-Ni%C3%B1os-Ni%C3%B1as-y-Adolescentes-NNA.pdf
10. Advisory Committee on Immunization Practices (ACIP). Contraindications and Precautions [Internet]. CDC. 2020 [citado 12 de Agosto de 2020]. Disponible en: https://www.cdc.gov/vaccines/hcp/acip-recs/general-recs/contraindications.html
11. .Meeting highlights from the Pharmacovigilance Risk Assessment Committee (PRAC) 29 November - 2 December 2021 [Consultada: 04/05/2021] Disponible en: https://www.ema.europa.eu/en/news/meeting-highlights-pharmacovigilance-risk-assessment-committee-prac-29-november-2-december-2021

CoronaVac

O que a ciência comprova

6.5. CoronaVac protegeu crianças a partir de 3 anos durante surto da ômicron em Xangai, mostra estudo chinês

Um estudo chinês conduzido em março de 2022, durante o surto da variante ômicron do SARS-CoV-2 em Xangai, mostrou mais uma vez que a CoronaVac é segura e protege crianças contra a Covid-19. Entre as crianças infectadas que manifestaram sintomas, mais de 70% ainda não tinham se vacinado, o que reforça a importância da imunização para prevenir desfechos mais graves. O artigo foi publicado na plataforma de preprints MedRxiv e conduzido por infectologistas do Hospital Pediátrico da Universidade Fudan, em Xangai.

Participaram da pesquisa 376 crianças e adolescentes com até 17 anos (média de 5 anos), que chegaram a ser atendidas no hospital da universidade chinesa. Deste grupo, 250 ainda não haviam se vacinado, 110 haviam tomado duas doses da CoronaVac e 16 haviam recebido apenas uma dose.
A análise mostrou que, dos 257 casos sintomáticos, 75% eram de crianças não vacinadas. A infecção sintomática foi mais frequente no grupo com idade menor que 3 anos (90/104), seguido do grupo de 3 a 5 anos (65/94) – apenas 5,3% das crianças nessa faixa etária haviam completado a imunização. Já entre as crianças de 6 a 17 anos, 59% estava vacinada, o que conferiu maior proteção para esse público.

A conclusão dos especialistas é que a alta cobertura vacinal contra a Covid-19, mesmo durante o surto da ômicron, reduziu o risco de uma infecção grave no público pediátrico. "A vacinação em massa das crianças de 3 a 17 anos começou em agosto de 2021. Mais de 70% das crianças nessa faixa etária já haviam sido vacinadas com as duas doses até o final de março de 2022."

Eles acrescentam que intervenções não farmacológicas, como uso de máscara em lugares fechados e higienização das mãos, combinadas com estratégias de vacinação, são críticas para prevenir a infecção e a doença grave e para mitigar a transmissão do SARS-CoV-2 na população pediátrica.

Os resultados da pesquisa reafirmam a eficácia do uso da CoronaVac em crianças a partir dos 3 anos, o que já havia sido comprovado por um amplo estudo conduzido no Chile. O trabalho feito com 500 mil crianças que tomaram CoronaVac durante o surto da ômicron demonstrou que a vacina do Butantan e da Sinovac tem efetividade de 69% contra internação em Unidade de Terapia Intensiva (UTI) e 64,6% contra hospitalização pela Covid-19.

Publicado em: 2/5/2022

Epidemiological and clinical features of SARS-CoV-2 Infection in children during the outbreak of Omicron Variant in Shanghai, March 7-March 31, 2022

Xiangshi Wang [1,†], Hailing Chang [1,†], He Tian [1,†], Jingjing Li [1], Zhongqiu Wei [1], Yixue Wang [2], Aimei Xia[1], Yanling Ge[1], Jiali Wang [1], Gongbao Liu [3], Jiehao Cai [1], Jianshe Wang [1], Qirong Zhu [1], Yanfeng Zhu [1,*], Xiaowen Zhai [4,*], Mei Zeng[1,*]

[1] Department of infectious diseases, Children's Hospital of Fudan University, National Children's Medical Center

[2] Department of intensive care medicine, Children's Hospital of Fudan University, National Children's Medical Center

[3] Department of medical affairs, Children's Hospital of Fudan University, National Children's Medical Center

[4] Department of Hematology, Children's Hospital of Fudan University, National Children's Medical Center

† Xiangshi Wang, Hailing Chang and He Tian equally contributed to this paper.

* Address for correspondence:

Yanfeng Zhu, MD, PhD, Department of Infectious Diseases, Children's Hospital of Fudan University, 399 Wanyuan Road, Shanghai 201102, China. E-mail: smilingyf@sina.com, Tel: 86-021-64931132 (office), 86-13916913420 (mobile).

Xiaowen Zhai, MD, PhD, Department of Hematology, Children's Hospital of Fudan University, 399 Wanyuan Road, Shanghai 201102, China. E-mail: zhaixiaowendy@163.com, Tel: 86-021-64931902 (office), 86-18017590808(mobile).

Mei Zeng, MD, PhD, Department of Infectious Diseases, Children's Hospital of Fudan

University, 399 Wanyuan Road, Shanghai 201102, China. E-mail: zengmeigao@163.com, Tel: 86-021-64931132 (office), 86-15000284050 (mobile).

Abstract

Objectives: To understand the epidemiological and clinical characteristics of pediatric SARS-CoV-2 infection during the early stage of Omicron variant outbreak in Shanghai.

Methods: This study included local COVID-19 cases <18 years in Shanghai referred to the exclusively designated hospital by the end of March 2022 since emergence of Omicron epidemic. Clinical data, epidemiological exposure and COVID-19 vaccination status were collected. Relative risks (RR) were calculated to assess the effect of vaccination on symptomatic infection and febrile disease.

Results: A total of 376 pediatric cases of COVID-19 (median age:6.0±4.2 years) were referred to the designated hospital during the period of March 7-31, including 257 (68.4%) symptomatic cases and 119 (31.6%) asymptomatic cases. Of the 307 (81.6%) children;3 years eligible for COVID-19 vaccination, 110 (40.4%) received 2-dose vaccines and 16 (4.0%) received 1-dose vaccine. The median interval between 2-dose vaccination and infection was 3.5 (IQR: 3, 4.5) months (16 days-7 months). Two-dose COVID-19 vaccination reduced the risks of symptomatic infection and febrile disease by 35% (RR 0.65, 95% CI:0.53-0.79) and 33% (RR 0.64, 95% CI: 0.51-0.81). Two hundred and sixteen (83.4%) symptomatic cases had fever (mean duration: 1.7±1.0.8 days), 104 (40.2%) had cough, 16.4% had transient leukopenia; 307 (81.6%) had an epidemiological exposure in household (69.1%), school (21.8%) and residential area (8.8%).

Conclusion: The surge of pediatric COVID-19 cases and multiple transmission model reflect wide dissemination of Omicron variant in the community. Asymptomatic infection is common among

46 Omicron-infected children. COVID-19 vaccination can offer protection against symptomatic infection

47 and febrile disease.

Introduction

The COVID-19 pandemic has caused devastation to the world's population, resulting in more than 6 million deaths as of April 20, 2022. SARS-CoV-2 infection in most pediatric cases is mild as compared to adults and the direct effect on child health is limited [1]. However, the indirect impacts on child medical care, education and mental health are considerable owing to lockdown, disruption of essential health service delivery, prolonged school closure and isolation [2,3]. The continuous genetic evolution of SARS-CoV-2 virus results in the emergence of multiple new variants of concern (VOC), which are associated with enhanced transmissibility or increased virulence and immune escape [4]. The Omicron variant, which was detected in November 2021 and almost replaced Delta variant by the end of January 2022, has led to the fifth global wave of COVID-19 epidemic [5]. The significant rise of pediatric infection was reported in the United States with children aged <18 years, representing 17.0%-19.0% of all cases during the Omicron period since late December 2021 [6,7].

Pediatric COVID-19 cases only accounted for a small proportion of infection in the early stages of the COVID-19 pandemic when many countries implemented non-pharmaceutical interventions and strict containment measures [8-12]. However, the incidence rate of COVID-19 in children showed a rising trend in the epidemic countries following suspension of lockdown and school reopening [12,13]. After the large-scale epidemic in early 2020, China entered a normalization stage of prevention and control, and massive COVID-19 vaccination campaign was launched nationwide in 2021. Inactivated SARS-CoV-2 vaccine BBIBP-CorV (by Sinopharm) and CoronaVac (by Sinovac) were approved for emergency use in children 3-17 years on June 2021 and COVID-19 vaccination program was initiated in pediatric population since late July 2021 across China. From May 2020, local pediatric COVID-19 infection linked to sporadic and cluster transmission were occasionally reported in China until the community outbreak

of Omicron variant appeared in Hong Kong Special Administrative Region since January 6, 2022 and subsequently in Shanghai since early March 2020 [14]. Omicron variant spread rapidly in Shanghai by the end of March and led to a surge of pediatric COVID-19 cases citywide. Here, we describe epidemiological and clinical characteristic of Omicron variant infections in Shanghainese children during the early stage of the outbreak.

Subject and Method

Subject

In this study, we included local COVID-19 cases <18 years of age who were notified in Shanghai and admitted to the exclusively designated hospital in Shanghai by the end of March 2022. Prior to 28 March, all pediatric COVID-19 cases notified in Shanghai were referred to the designated hospital for concentrating management and isolation. Since 28 March when a large number of cases were confirmed by massive screening test, most of asymptomatic and mild pediatric cases aged 5-17 years were almost sent to Fangcang shelter hospitals. All confirmed cases irrespective of symptoms are required for in-hospital isolation and are discharged if the cycle threshold (Ct) value for the viral nucleic acid is great than 35 on PCR test for the two consecutive respiratory samples taken 24-hour apart [15].

Case definition and classification

All COVID19 cases were laboratory-confirmed by the Shanghai CDC reference laboratory using real-time RT-PCR commercial kit. The Ct value <40 was defined as a positive nucleic acid amplification test. COVID-19 cases were classified as asymptomatic and symptomatic cases. Symptomatic cases were classified as mild, moderate and severe cases. An asymptomatic case is defined as a person with a positive nucleic acid test but without any clinical symptom of COVID-19. A confirmed symptomatic case is

defined as a person presenting clinical signs and symptoms of COVID-19. COVID-19 disease severity classification is based on the WHO guidance [16]. Pneumonia was diagnosed based on clinical signs (fever and/or cough accompanying with one of the following signs: moist rales on auscultation, difficulty breathing/dyspnoea, fast breathing, chest indrawing), radiological findings compatible with pneumonia.

Data collection

Data were collected via a face-to-face interview with parents or teenagers and electronic medical chart, including: demographic information, epidemiological exposure setting, COVID-19 vaccination status on dose and date, clinical symptoms, laboratory findings and chest imaging if examined, treatment and outcome. Informed consent from parents was not required by the ethics committee because all data were de-identified and not involved in personal privacy.

Statistical analysis

Data was entered into Excel version 2016 (Microsoft, Redmond, Washington) for analysis and the statistical analysis was preformed using SPSS (IBM Statistic 23.0). Categorical variables are described as absolute numbers and percentage. Continuous variables with normal distribution are expressed as mean ± standard deviation. Median (25% to 75% interquartile range (IQR) are used when the frequency distributions were skewed. Differences between groups are compared using Mann-Whitney *U*-test and Student's *t*-test as appropriate. A difference with $P < 0.05$ is considered to be statistically significant. Relative risks (RR) were calculated using proportion of symptomatic infection and febrile cases by vaccination status, with the referent group being ≥2-dose vaccinees.

Results

Demographic characteristics

The first local pediatric case was notified on March 2022 and increased remarkably from 14 March onwards (as shown in Figure 1). As of 31 March, a total of 376 pediatric cases of COVID-19 were

115 referred to the exclusively designated hospital. The ratio of male-to-female was 1.1 (206/170). The 376
116 cases were aged 11 days-17 years with the median age of 5.0 years (IQR: 2, 9) and the mean age of
117 6.0±4.2 years: 28 (7.4%) cases in age group <1 year, 76 (20.2%) cases in age group 1-2 years, 94 (25.0%)
118 cases in age group 3-5 years, 134 (35.6%) cases in age group 6-11 years, and 44 (11.7%) cases in age
119 group ≥12 years.

120 **Epidemiological exposure**

121 Three hundred and seven (81.6%) cases had a clear history of exposure, of whom, 213 (69.1%)
122 had a close contact with confirmed adult cases in household, 67 (21.8%) had a clear contact with
123 confirmed child cases in school, and 27 (8.8%) had an epidemiological linkage to residential area
124 where cluster cases of COVID-19 were reported. As shown in Figure 2, the first child case acquired
125 infection in family, soon after, child cases linked to possible community transmission were found, who
126 had no clear exposure.

127 **Vaccination status**

128 A total of 126 had received at least one dose of an inactivated COVID-19 vaccine, accounting for
129 33.5% of the total 376 pediatric cases and 46.3% of the 272 pediatric cases aged ≥3 years eligible for
130 COVID-19 vaccination in China. Of the 272 vaccine-eligible children, 146 (53.6%) were unvaccinated,
131 110 (40.4%) had received 2 doses and 16 (4.0%) had received 1 dose. Among the 94 preschool children
132 aged 3-5 years, the proportions of 1 dose and 2 doses of COVID-19 vaccination were 3.2% (3/94) and
133 5.3% (5/94), respectively. Among the 178 school children aged 6-17 years, the proportions of 1 dose and
134 2 doses of COVID-19 vaccination were 7.3% (13/174) and 59.0% (105/174), respectively. Overall, the
135 interval between vaccination and breakthrough infection ranged from 16 days to 7 months (median: 3.5
136 (IQR: 3, 4.5) months).

As shown in table 1, 2-dose COVID-19 vaccination reduced the risk of symptomatic infection and febrile disease by 35% (0.65, 95% CI: 0.53-0.79) and by 36% (RR 0.64, 95% CI: 0.51-0.81) in children 0-17 years, by 29% (RR 0.71, 95% CI: 0.57-0.88) and 29% (RR 0.71, 95% CI: 0.55-0.92) in children 3-17 years eligible for COVID-19 vaccine. However, one-dose vaccination could not significantly decrease the relative risks of symptomatic infection and febrile disease.

Clinical manifestation and course

Of the 376 cases, 257 (68.4%) presented symptoms and 119 (31.6%) had no symptoms before and duration hospitalization. Of the 257 symptomatic cases, 216 (83.4%) experienced fever (axillary temperature >37.5°C) with a mean fever spike of 38.9± 0.6°C (range: 37.6-41°C) and a mean fever duration of 1.7±1.0.8 days (range: 0.5-4 days), 104 (40.2%) presented cough, 28 (10.8%) self-reported sore throat, 13 (5.0%) self-reported stuffy nose, 6 (2.3%) had runny nose, 11 (4.2%) had nausea or vomiting or diarrhea, 2 (0.8%) self-reported transient loss of taste and smell. No severe case was diagnosed. Twenty five cases had chest CT performed due to fever >38.5°C lasting for 3 days or cough worsening after admission or routine examination prior to the referral. The chest images showed patchy infiltrates or ground-glass opacity in 4 cases and one of them was right lung lobar pneumonia caused by Mycoplasma pneumoniae. Six (1.6%) cases had comorbidity including brain tumor, febrile seizure, psychomotor retardation, hemophilia, Henoch-Schonlein purpura, and cardiac arrhythmia in each.

As shown in table 2, 22.8% (57/250) of unvaccinated cases were asymptomatic while 50.0% (55/110) of 2-dose vaccinated cases were asymptomatic (P=0.000); 65.2% (163/250) of unvaccinated cases were febrile while 41.8%% (46/110) of 2-dose vaccinated cases were febrile (P=0.000). Symptomatic infection was significantly frequently seen in the age group <3 years than in the age group 3-5 years (P=0.003) and 6-17 years (P=0.000). Fever was significantly frequently seen in the age group

3-5 years than in the age group <3 years (P=0.000) and 6-17 years (P=0.005).

Of the 225 case who had complete peripheral blood cell count tested, 37 (16.4%) had white blood cell (WBC) count $<4\times10^9$/L, 173 (76.9%) had WBC count $4\text{-}9\times10^9$/L, 13 (5.8%) had WBC count $10\text{-}14\times10^9$/L and 2 (7.1%) had WBC count $\geq15\times10^9$/L. The WBC count ranged from 1.9×10^9/L to 15.5×10^9/L. No thrombopenia was observed. Of the 187 cases who had peripheral blood C-reactive protein (CRP) tested, 178 (95.2%) had CRP <8 mg/L, 8 (4.3%) had CRP >8 mg/L (range: 8.8-35.8 mg/L) and 1 (0.5%) had CRP 56 mg/L who had co-infection with *mycoplasma pneumoniae* and developed typical lobar pneumonia in right lung. Of the 196 cases who had serum biochemical markers and 8 (4.1%) showed slightly elevated liver enzyme.

For symptomatic cases, Ibuprofen and or Chinese traditional medicines were prescribed depending on the personalized condition and medication compliance. Only one case who had a clear diagnosis of mycoplasma pneumonia was prescribed antibiotics. All cases were discharged when the Ct value of the nucleic acid of SARS-CoV-2 virus reached >35. The average duration of Ct value of the nucleic acid of SARS-CoV-2 virus >35 since admission was 11.7±3.7days (range: 3-25 days; symptomatic verse asymptomatic: 11.7±3.6 verse 11.7±3.9, P=0.064).

Discussion

This study first presents the epidemiological and clinical profiles of Omicron variant infection in localized children during the early phase of outbreak in Shanghai. As of 31 March 2022, all pediatric COVID-19 cases were mild (68.4%) or asymptomatic (31.6%). However, a few of severe pediatric cases were reported during the period of COVID-19 outbreak in Wuhan in early 2020 [17]. Moreover, the proportion of asymptomatic cases was 2-time more than that seen in the Wuhan outbreak. We reason that

high coverage of COVID-19 vaccination among Shanghainese children is very likely to lower the risk of severe Omicron infection-associated disease. The mass COVID-19 vaccination roll-out among children 3-17 years started between Mid-Aug 2021 and December 2021 in Shanghai and the estimated coverage rate of 2-dose COVID-19 vaccination among children 3-17 years has exceeded 70% by of the end of March in 2022. In this case cohort 46.3% of children eligible for COVID-19 vaccination prior to 16 days to 7 months (median: 3.5months). Observational studies from some countries with the high levels of population immunity generated by natural infection or vaccine have shown receipt of two doses of COVID-19 vaccines and a booster dose can offer protection against symptomatic and severe Omicron infection in a short-term period of vaccination [4,18-22].

Current evidences consistently show a reduction in neutralizing antibody against Omicron in serum of convalescent or vaccinated individuals, resulting in Omicron's immune escape potential against vaccine- and infection-induced immunity [4,23]. However, two recent study based on real-world observation among children showed the modest effectiveness for COVID-19 vaccine against Omicron infection [25,26]. Based on our findings, receipt of 2-dose inactivated COVID-19 vaccine within 17 days to 7 months after fully primary vaccination potentially reduced the risk of symptomatic Omicron infection by 31% and febrile disease by 59% in children. We did not estimate vaccine protection against severe infection because no severe COVID-19 cases were diagnosed. There is also evidence of waning of vaccine effectiveness over time of the primary series against infection and symptomatic disease for the studied vaccines. However, the vaccine effectiveness against Omicron infection and disease can be restored and increase to > 40% to 80% within a short follow-up time after a third booster dose in studies from five countries (United Kingdom, Denmark, Canada, South Africa, USA) [4]. Ten cases of reinfection with Omicron variant were identified within 23 to 87 days of a previous Delta infection was

reported in the USA and most were pediatric cases [26]. Thus, eligible children and adolescents should remain up to date with recommended COVID-19 vaccination in response to Omicron outbreak. So far, a third booster dose of COVID-19 vaccine has been recommended for use in adults but not in children in China. In light of the field findings, a booster dose should also be recommended for eligible children 3-17 years.

We observed that school children aged 6-11 years comprised the most cases, followed by home-care children <2 years and preschool children 3-5 year. The distribution of age groups in the early stage of outbreak reflects the cluster transmission of COVID-19 centered in elementary school, kindergarten, and household. Of note, age group 12-17 years accounted for the smallest proportion of pediatric cases, among which, the high coverage rate of COVID-19 vaccination was as high as 95%. Based on the epidemiological investigation, more than 80% children had a clear history of exposure, mostly occurring in family (69.1%) and school (21.8%), occasionally in residential area (8.8%). The remaining 18.4% of children had no clear contact with confirmed cases, reflecting small-scale community transmission had already appeared prior to the large-scale outbreak since April. During the 2020 outbreak of COVID-19, 80%-90% of confirmed child cases were family cluster cases and community transmission was unusual in China [17,27]. Rapid increases in pediatric COVID-19 cases and epidemiological unrelated cases also suggest the occurrence of high community transmission of Omicron variant in Shanghai since the early epidemic wave.

We observed most of localized pediatric cases (83.4%) of symptomatic Omicron infection presenting fever. However, fever is less commonly seen in pediatric COVID-19 cases reported in China (58%) and the USA (56%) during the first wave of pandemic in 2020 [10, 17]. Fever could be helpful for early recognition and diagnosis of COVID-19 because parents always worry about the febrile child and

visit hospital for seeking medical care. The febrile course of Omicron infection is brief with a mean fever duration of 1.7±1.0.8 days, significantly shorter than fever duration seen influenza (4 days) [28]. The febrile duration is helpful to differentiate COVID-19 from influenza in children when the epidemics of COVID-19 and influenza overlap.

The potential role in transmission for most asymptomatic and mild child cases should not be neglected. A study showed that symptomatic and asymptomatic children can carry high quantities of live SARS-CoV-2, creating a potential reservoir for transmission [29]. Vaccinees with mild or asymptomatic Omicron infection shed infectious virus 6-9 days after onset or diagnosis, even after symptom resolution [30]. In fact, asymptomatic infection in children was underestimated in the early stage of outbreak because massive screening of COVID-19 cases had not been carried out before 28 March. After citywide large-scale screening, notifiable asymptomatic cases accounted for 90% more or less in April. Asymptomatic infection was much more common in vaccinated children than in unvaccinated children (50% vs 22.8%). Vaccination can offer protection against symptomatic infection and febrile disease, on the other hand, the role of asymptomatic children play in viral transmission is of attention during outbreak. High prevalence of asymptomatic infection is likely a major factor in the widespread of the Omicron variant among population.

In summary, COVID-19 is mild and subtle in Shanghainese children with the high level of vaccine-induced immunity during the early stage of Omicron outbreak. COVID-19 vaccination can offer partial protection against symptomatic COVID-19. Ongoing Omicron epidemic will increase the risk of exposure among children with underlying medical conditions, who are usually unvaccinated, therefore, severe COVID-19 infection is anticipated to be encountered in children. Non-pharmaceutical interventions in combination with vaccination strategies are critical to prevent infection and severe

247 disease and to mitigate the impact of COVID-19 in pediatric population.

248 **Transparency declaration**

249 The authors have declared that there are no conflicts of interest in relation to this work. Disclosure forms

250 provided by the authors are available as Supplementary material.

251 *Funding*

252 This work was supported by the Science and Technology Commission of Shanghai Municipality (NO.

253 20JC141020002), the Key Development Program of Children's Hospital of Fudan University

254 (EK2022ZX05), the Three-Year Action Plan for Strengthening the Construction of Public Health System

255 in Shanghai (2020-2022) (GWV-3.2), and the Key Discipline Construction Plan from Shanghai

256 Municipal Health Commission (GWV-10.1-XK01). None of the funding organizations played any role

257 in the trial design, data collection, analysis of results, or writing of the manuscript.

258 **Author contributions**

259 Conceptualization: YZ, XZ, MZ; Methodology: XW, HC, HT, MZ; Software: XW, HC, HT; Validation:

260 JC; Formal Analysis: XW, HC, HT, YZ, MZ; Investigation: JL, ZW, YW, AX, YG, JW, GL, JC; Writing-

261 Original Draft: XW, HC, YZ, MZ; Writing-Review & Editing: XW, MZ; Visualization: JC, HC;

262 Supervision: YZ, GL, JW, QZ, XZ, MZ; Project Administration: XZ; Funding acquisition: XZ, MZ. All

263 authors approved the manuscript for publication.

264

265 **Reference**

266 1. World Health Organization. COVID-19 disease in children and adolescents: Scientific brief, 29

267 September 2021. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-Sci_Brief-

268 Children_and_adolescents-2021.1. [Accessed 18 April 2022].

2. World Health Organization. Pulse survey on continuity of essential health services during the COVID-19 pandemic: interim report, 27 August 2020. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-EHS_continuity-survey-2020.1. [Accessed 18 April 2022].

3. Meherali S, Punjani N, Louie-Poon S, Abdul Rahim K, Das JK, Salam RA, et al. Mental Health of Children and Adolescents Amidst COVID-19 and Past Pandemics: A Rapid Systematic. Review Int J Environ Res Public Health 2021;18(7):3432. https://doi.org/10.3390/ijerph18073432

4. World Health Organization. Enhancing response to Omicron SARS-CoV-2 variant: Technical brief and priority actions for Member States. Available at: https://www.who.int/publications/m/item/enhancing-readiness-for-omicron-(b.1.1.529)-technical-brief-and-priority-actions-for-member-states. [Accessed 22 April 2022].

5. World Health Organization. Weekly epidemiological update on COVID-19 - 1 March 2022. Available from: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---1-march-2022. [Accessed 22 April 2022].

6. Iuliano AD, Brunkard JM, Boehmer TK, Peterson E, Adjei S, Binder AM, et al. Trends in Disease Severity and Health Care Utilization During the Early Omicron Variant Period Compared with Previous SARS-CoV-2 High Transmission Periods - United States, December 2020-January 2022. MMWR Morb Mortal Wkly Rep 2022;71(4):146-52. https://doi.org/10.15585/mmwr.mm7104e4

7. American Academy of Pediatrics, Children's Hospital Association. Children and COVID-19: State Data Report (Version3/31/22). Available from: https://www.aap.org/en/pages/2019-novel-coronavirus-covid-19-infections/children-and-covid-19-state-level-data-report/ [Accessed 22 April 2022].

8. World Health Organization (WHO). 2020. Report of the WHO-China joint mission on coronavirus disease 2019 (covid-19). Available from: https://www.who.int/publications/i/item/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19). [Accessed 21 April 2022].

9. Choi SH, Kim HW, Kang JM, Kim DH, Cho EY. 2020. Epidemiology and clinical features of coronavirus disease 2019 in children. Clin Exp Pediatr 63;(4):125-32. https://doi.org/10.3345/cep.2020.00535

10. CDC COVID-19 Response Team. Coronavirus Disease 2019 in Children - United States, February 12-April 2, 2020. MMWR Morb Mortal Wkly Rep 2020;69(14):422-6. https://doi.org/10.15585/mmwr.mm6914e4

11. Ladhani SN, Amin-Chowdhury Z, Davies HG, Aiano F, Hayden I, Lacy J, et al. COVID-19 in children: analysis of the first pandemic peak in England. Arch Dis Child 2020;105(12):1180-5. https://doi.org/10.1136/archdischild-2020-320042

12. Malcangi G, Inchingolo AD, Inchingolo AM, Piras F, Settanni V, Garofoli G, et al. COVID-19 Infection in Children and Infants: Current Status on Therapies and Vaccines. Children (Basel). 2022;9(2):249. https://doi.org/10.3390/children9020249

13. Singhal T. The Emergence of Omicron: Challenging Times Are Here Again! Indian J Pediatr 2022;89(5):490-6. https://doi.org/10.1007/s12098-022-04077-4

14. Smith DJ, Hakim AJ, Leung GM, Xu WB, Schluter WW, Novak RT, et al. Preplanned Studies: COVID-19 Mortality and Vaccine Coverage - Hong Kong Special Administrative Region, China, January 6, 2022–March 21, 2022. China CDC Weekly 2022; 4(14): 288-92. https://doi.org/10.46234/ccdcw2022.071

15. National Health Commission of The People's Republic of China. Interim protocol of diagnosis and

313 treatment of 2019 novel coronavirus-associated pneumonia (the nineth version). Available from:
314 http://www.gov.cn/zhengce/zhengceku/2022-03/15/content_5679257.htm. [Accessed 22 April 2022].
315 16. World Health Organization. Living guidance for clinical management of COVID-19. Available at:
316 https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-2. [Accessed 22 April 2022].
317 17. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J, et al. Chinese Pediatric Novel Coronavirus Study
318 Team. SARS-CoV-2 Infection in Children. N Engl J Med 2020;382(17):1663-5.
319 https://doi.org/10.1056/NEJMc2005073
320 18. Cloete J, Kruger A, Masha M, du Plessis NM, Mawela D, Tshukudu M, et al. Paediatric
321 hospitalisations due to COVID-19 during the first SARS-CoV-2 omicron (B.1.1.529) variant wave
322 in South Africa: a multicentre observational study. Lancet Child Adolesc Health 2022;6(5):294-302.
323 https://doi.org/10.1016/S2352-4642(22)00027-X
324 19. Wang L, Berger NA, Kaelber DC, Davis PB, Volkow ND, Xu R. Incidence Rates and Clinical
325 Outcomes of SARS-CoV-2 Infection with the Omicron and Delta Variants in Children Younger than
326 5 Years in the US. JAMA Pediatr 2022;1:e220945.
327 https://doi.org/10.1001/jamapediatrics.2022.0945
328 20. I Iuliano AD, Brunkard JM, Boehmer TK, Peterson E, Adjei S, Binder AM, et al. Trends in Disease
329 Severity and Health Care Utilization During the Early Omicron Variant Period Compared with Previous
330 SARS-CoV-2 High Transmission Periods - United States, December 2020-January 2022. MMWR Morb
331 Mortal Wkly Rep 2022;71(4):146-52. https://doi.org/10.15585/mmwr.mm7104e4
332 21. Menni C, Valdes AM, Polidori L, Antonelli M, Penamakuri S, Nogal A, et al. Symptom
333 prevalence, duration, and risk of hospital admission in individuals infected with SARS-CoV-2
334 during periods of omicron and delta variant dominance: a prospective observational study from the

335 ZOE COVID Study. Lancet 2022;7:S0140-6736(22)00327-0. https://doi.org/10.1016/S0140-
336 6736(22)00327-0
337 22. Wolter N, Jassat W, Walaza S, Welch R, Moultrie H, Groome M, et al. Early assessment of the
338 clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study. Lancet
339 2022;399(10323):437-46. https://doi.org/10.1016/S0140-6736(22)00017-4
340 23. Peiris M, Cheng S, Mok CKP, Leung Y, Ng S, Chan K, et al. Neutralizing antibody titres to SARS-
341 CoV-2 Omicron variant and wild-type virus in those with past infection or vaccinated or boosted with
342 mRNA BNT162b2 or inactivated CoronaVac vaccines. Res Sq [Preprint] 2022;rs.3.rs-1207071.
343 https://doi.org/10.21203/rs.3.rs-1207071/v1
344 24. Fowlkes AL, Yoon SK, Lutrick K, Gwynn L, Burns J, Grant L, et al. Effectiveness of 2-Dose
345 BNT162b2 (Pfizer BioNTech) mRNA Vaccine in Preventing SARS-CoV-2 Infection Among
346 Children Aged 5-11 Years and Adolescents Aged 12-15 Years - PROTECT Cohort, July 2021-
347 February 2022. MMWR Morb Mortal Wkly Rep. 2022 Mar 18;71(11):422-8.
348 https://doi.org/10.15585/mmwr.mm7111e1
349 25. Araos R, Jara A, Undurraga E, Zubizarreta J, Gonzalez C, Acevedo J, et al. Effectiveness of
350 CoronaVac in children 3 to 5 years during the omicron SARS-CoV-2 outbreak. Research Square
351 [Preprint]. https://doi.org/10.21203/rs.3.rs-1440357/v1
352 26. Roskosky M, Borah BF, DeJonge PM, Donovan CV, Blevins LZ, Lafferty AG, et al. Notes
353 from the Field: SARS-CoV-2 Omicron Variant Infection in 10 Persons Within 90 Days of Previous
354 SARS-CoV-2 Delta Variant Infection - Four States, October 2021-January 2022. MMWR Morb
355 Mortal Wkly Rep 2022;71(14):524-6. https://doi.org/10.15585/mmwr.mm7114a2
356 27. Jiehao C, Jin X, Daojiong L, Zhi Y, Lei X, Zhenghai Q, et al. A Case Series of Children With

2019 Novel Coronavirus Infection: Clinical and Epidemiological Features. Clin Infect Dis 2020;71(6):1547-51. https://doi.org/10.1093/cid/ciaa198

28. Wang X, Cai J, Yao W, Zhu Q, Zeng M. Socio-economic impact of influenza in children:a single-centered hospital study in Shanghai. Chin J Epidemiol 2015;36(1):27-30.

29. Yonker LM, Boucau J, Regan J, Choudhary MC, Burns MD, Young N, et al. Virologic Features of Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Children. J Infect Dis 2021;224(11):1821-9. https://doi.org/10.1093/infdis/jiab509

30. Takahashi K, Ishikane M, Ujiie M, Iwamoto N, Okumura N, Sato T, et al. Duration of Infectious Virus Shedding by SARS-CoV-2 Omicron Variant-Infected Vaccinees. Emerg Infect Dis 2022;28(5):998-1001. https://doi.org/10.3201/eid2805.220197

368 Table 1. Clinical characteristics of SARS-CoV-2 infection according to COVID-19 vaccination
369 status

Vaccination status by age group (years)	Symptomatic case, n (%)	Relative risk (95% CI)	Febrile cases n (%)	Relative risk (95% CI)
0-17 (n=376)				
unvaccinated (n=250)	193 (77.2%)	ref	163 (65.2%)	ref
1 dose (n=16)	9 (56.3%)	0.73 (0.47-1.13)	7 (43.8%)	0.67 (0.38-1.18)
2 doses (n=110)	55 (50.0%)	0.65 (0.53-0.79)	46 (41.8%)	0.64 (0.51-0.81)
3-17 (n=272)				
unvaccinated (n=146)	103 (70.5%)	ref	86 (58.9%)	ref
1 dose (n=16)	9 (56.3%)	0.80 (0.51-1.24)	7 (43.8%)	0.74 (0.42-1.32)
2 doses (n=110)	55 (50.0%)	0.71(0.57-0.88)	46 (41.8%)	0.71 (0.55-0.92)

370 **Abbreviation:** ref = referent group.

371
372

Table 2. Clinical characteristics of SARS-CoV-2 virus infection by age group

Clinical characteristics	Total (n=376)	Age group (years)			*P* value	Vaccination status		*P* value
		<3 (n=104)	3-5 (n=94)	6-17 (n=178)		Unvaccinated (n=250)	2-dose vaccination (n=110)	
Asymptomatic cases, n (%)	119 (31.6%)	14 (13.5%)	29 (30.9%)	76 (42.7%)	0	57 (22.8%)	55 (50.0%)	0
Symptomatic cases, n (%)	257 (68.4%)	90 (86.5%)	65 (69.1%)	102 (57.3%)	0	193 (77.2%)	55 (50.0%)	0
Febrile cases, n (%)	216 (57.4%)	44 (42.3%)	59 (62.8%)	80 (44.9%)	0.006	163 (65.2%)	46 (41.8%)	0
Fever spike (°C), mean±SD	38.9±0.6	39.0±0.7	39.0±0.6	38.8±0.6	0.064	38.9±0.6	38.8±0.7	0.27
Fever duration (days), mean±SD	1.7±0.8	1.7±0.9	1.6±0.6	1.8±0.8	0.45	1.6±0.8	1.9±0.9	0.19

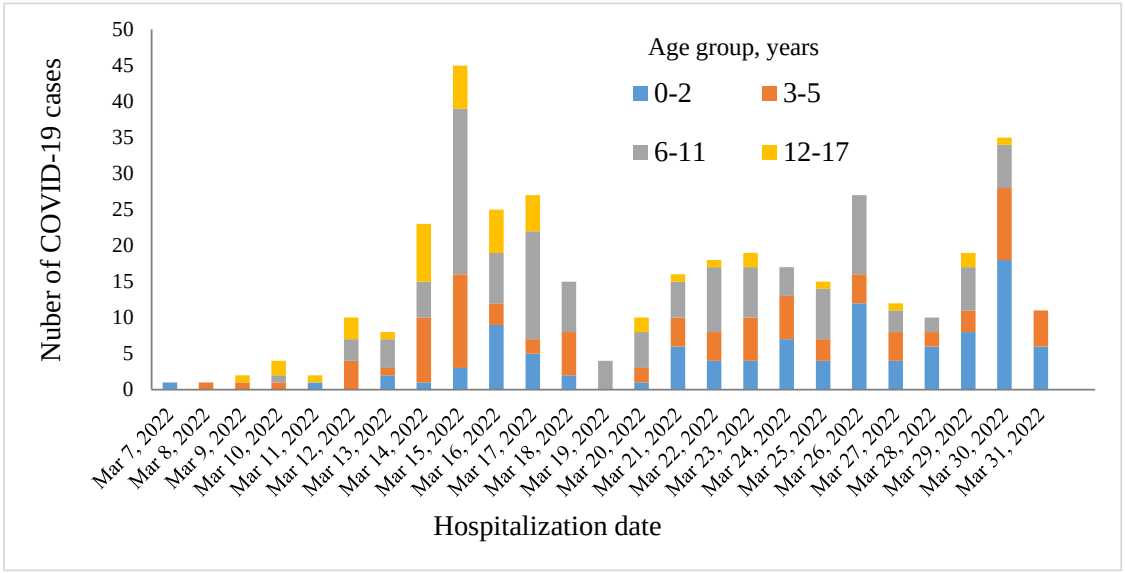


Figure 1. Daily COVID-19 cases referred to the designated hospital for children aged <18 years from March 1 to March 31, 2022

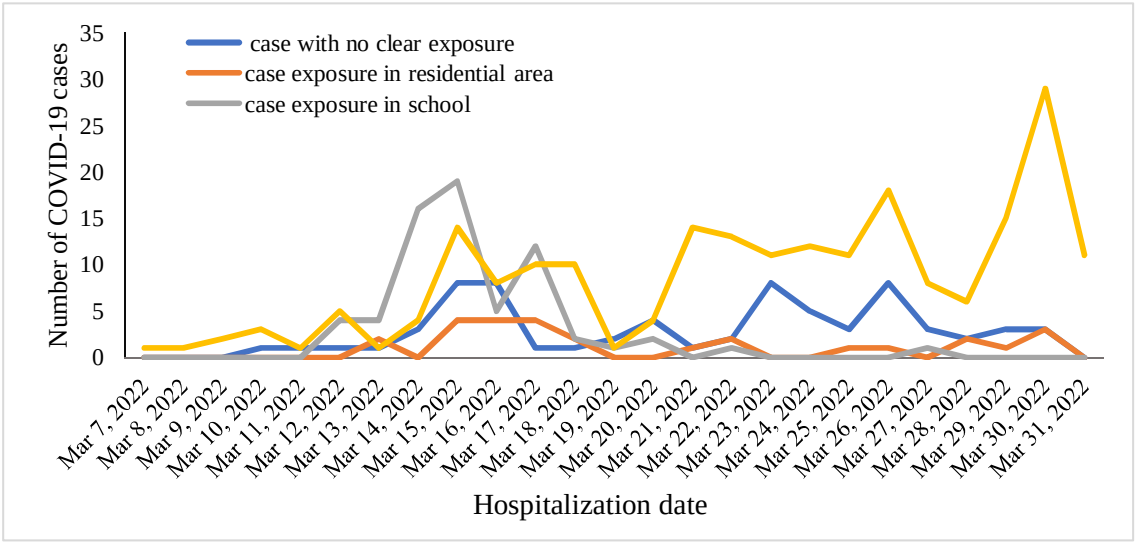


Figure 2. Model of epidemiological exposure over time among pediatric COVID-19 cases

6.6. CoronaVac é 69% eficaz contra internação por Covid-19 de crianças de 3 a 5 anos, diz estudo chileno feito durante surto de ômicron

Uma pesquisa realizada com 500 mil crianças que tomaram a CoronaVac durante o surto de ômicron no Chile demonstrou que a vacina do Butantan e da Sinovac tem eficácia de 69% contra internação em Unidade de Terapia Intensiva (UTI), 64,6% contra hospitalização pela Covid-19 e 38,2% contra a infecção.

O estudo foi publicado na plataforma de pré-prints Reserch Square e ainda precisa de revisão de pares.

Evidências contra ômicron

O grupo de estudo incluiu 516.250 crianças de três a cinco anos filiadas ao Fundo Nacional de Saúde (FONASA), o sistema público de saúde do Chile. Destas, 490.694 receberam a CoronaVac e as demais do grupo controle não receberam vacina. Foram excluídas do estudo crianças com teste positivo para Covid-19.

As crianças tomaram duas doses da CoronaVac, com 28 dias de intervalo entre elas, entre 6/12/21 e 26/2/22, durante a campanha de imunização contra Covid-19 do país. Segundo a pesquisa, "as estimativas fornecem evidências da eficácia da vacinação em crianças de três a cinco anos durante o surto de ômicron no Chile".

Os pesquisadores lembram estimativas preliminares recentes da eficácia da vacinação de duas doses de CoronaVac em crianças de seis a 16 anos, em um período em que delta era a variante circulante predominante de SARS-CoV-2.

No estudo anterior, a eficácia estimada da CoronaVac foi de 74,5% para a prevenção de Covid-19, 91% para a prevenção de hospitalização e 93,8% para a prevenção de internação em UTI relacionada à Covid-19. As estimativas para o subgrupo de

crianças de seis a 11 anos foram de 75,8% para a prevenção contra Covid-19 e 77,9% para a prevenção de hospitalização pela doença.

"Enquanto as estimativas não são diretamente comparáveis, a menor eficácia estimada da vacina pode ser devido à ômicron ou porque a coorte incluiu crianças mais novas", descreveram os pesquisadores.

Os estudiosos reiteram que pesquisas recentes sugerem que as vacinas podem ser menos eficazes contra a ômicron e que estudos observacionais têm limitações. "Não temos dados para avaliar se crianças vacinadas e não vacinadas ou seus cuidadores diferem em algumas características não observáveis, como o cumprimento dos protocolos contra a Covid-19. Outra limitação em nosso estudo diz respeito às capacidades de vigilância genômica. A estratégia do Ministério da Saúde concentrou-se na detecção de variantes de preocupação por meio da vigilância do viajante e da comunidade, mas usa amostragem não probabilística", descreveu o estudo.

Publicado em: 15/03/2022

Artigo

Effectiveness of CoronaVac in children 3 to 5 years during the omicron SARS-CoV-2 outbreak

Rafael Araos (rafaelaraos@udd.cl)
Universidad del Desarrollo
Alejandro Jara
Ponitificia Universidad Catolica de Chile
Eduardo Undurraga
Ponitificia Universidad Catolica de Chile
Jose Zubizarreta
Harvard University
Cecilia Gonzalez
Ministerio de Salud, Gobierno de Chile
Johanna Acevedo
Ministerio de Salud, Gobierno de Chile
Alejandra Pizarro
Ministerio de Salud, Gobierno de Chile
Veronica Vergara
Ministerio de Salud, Gobierno de Chile
Mario Soto Marchant
Ministerio de Salud, Gobierno de Chile
Rosario Gilabert
Ministerio de Salud, Gobierno de Chile
Juan Carlos Flores
Pontificia Universidad Católica de Chile
Pamela Suarez
Ministerio de Salud, Gobierno de Chile
Paulina Leighton
Ministerio de Salud, Gobierno de Chile
Pablo Eguiguren
Ministerio de Salud, Gobierno de Chile
Juan Carlos Rios
Ministerio de Salud, Gobierno de Chile
Jorge Fernandez
Instituto de Salud Pública de Chile
Heriberto Garcia-Escorza

Instituto de Salud Pública de Chile

Brief Communication

Keywords: SARS-CoV-2, Covid-19, vaccine effectiveness, inactivated SARS-CoV-2 vaccine, pediatric cohort, Omicron

Posted Date: March 15th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1440357/v1

Abstract

The outbreak of the B.1.1.529 lineage of SARS-CoV-2 (omicron) has caused an unprecedented number of Covid-19 cases, including pediatric hospital admissions. Policymakers urgently need evidence of vaccine effectiveness in children to balance the costs and benefits of vaccination campaigns, but the evidence is sparse or non-existing. Leveraging a population-based cohort of 490,694 children aged 3–5 years, we estimated the effectiveness of administering a two-dose schedule, 28 days apart, of CoronaVac using inverse probability-weighted survival regression models to estimate hazard ratios of complete immunization over non-vaccination, accounting for time-varying vaccination exposure and relevant confounders. The study was conducted between December 6, 2021, and February 26, 2022, during the omicron outbreak in Chile. The estimated vaccine effectiveness was 38.2% (95%CI, 36.5–39.9) against Covid-19, 64.6% (95%CI, 49.6–75.2) against hospitalization, and 69.0% (95%CI, 18.6–88.2) to prevent intensive care unit admission. The effectiveness was modest; however, protection against severe disease remained high.

Main Text

The emergence and spread of the B.1.1.529 lineage of SARS-CoV-2, the cause of coronavirus disease 2019 (Covid-19), has caused an unprecedented number of infections worldwide in a short period.[1,2] Emerging evidence suggests that omicron causes less severe disease than previous variants of concern (VOC), probably due to lower virulence, infection-acquired immunity, and higher vaccination coverage.[3–6] However, its high transmissibility and ability to partially evade the immune response induced has been associated with a substantial increase in severe Covid-19 cases globally.[2] The absolute number of pediatric hospital admissions has also surpassed previous waves,[4,7,8] straining healthcare systems even further. The increase may be related to higher transmissibility of omicron, less use of facemasks in children, and, especially concerning, lower vaccination rates among children.

Policymakers urgently need evidence of the effectiveness of Covid-19 vaccines in preventing severe clinical presentations of Covid-19 in children to balance the costs and benefits of mass vaccination campaigns. While the risk of severe Covid-19 in healthy children is substantially lower than among adults, vaccinating children may reduce community transmission, avoid potentially life-threatening presentations such as multisystemic inflammatory syndrome (MIS-C), and prevent long-term consequences of SARS-CoV-2 infection.[9] Although numerous countries are vaccinating children, few have authorized Covid-19 vaccines for children under five, and some have restricted vaccines for children older than 12 years.[10] Evidence of the efficacy or effectiveness of Covid-19 vaccines in children is limited, primarily related to mRNA vaccines, and only one study was conducted during the omicron outbreak.[11–14] To the best of our knowledge, there is no evidence of vaccine effectiveness against Covid-19 in children under five years. Furthermore, recent research suggests that several Covid-19 vaccine platforms provide limited protection against infection and symptomatic disease caused by the omicron variant but were more effective against severe disease.[15–17] These studies have examined vaccine protection against omicron in adult

populations but are consistent with preliminary, unpublished results from a study in children 5 to 12 years.[13]

Leveraging a population-based cohort of children aged 3 to 5 years, we estimated the effectiveness of the complete primary immunization schedule (two doses, 28 days apart) of an inactivated SARS-CoV-2 vaccine, CoronaVac, to prevent laboratory-confirmed Covid-19, hospitalization, and admission to an intensive care unit (ICU). We estimated vaccine effectiveness using inverse probability-weighted survival regression models to estimate hazard ratios of complete immunization (starting 14 days after the second dose) over the unvaccinated status, accounting for time-varying vaccination exposure and available clinical, demographic, and socioeconomic confounders at baseline.

Our study cohort included 516,250 children aged 3 to 5 years affiliated to the Fondo Nacional de Salud (FONASA), the public national healthcare system. 490,694 children had received two doses of CoronaVac, 28 days apart between December 6, 2021, and February 26, 2022, or did not receive any Covid-19 vaccination. We excluded children who had probable or confirmed Covid-19 according to reverse-transcription polymerase-chain-reaction assay for SARS-CoV-2 or antigen test before December 6, 2021 (Supplementary Figure S1). The cohort characteristics are described in Supplementary Tables S1 and S2. Vaccination rollout was organized through a public schedule; children needed to show up at their nearest vaccination site with their national ID card (Supplementary Figure S2). The study period enclosed the omicron outbreak in Chile (Supplementary Tables S3 and S4, Fig.S3)

The estimated adjusted vaccine effectiveness for CoronaVac in children aged 3 to 5 years during the omicron outbreak was 38.2% (95% CI, 36.5–39.9) for the prevention of Covid-19, 64.6% (95% CI, 49.6–75.2) for the prevention of hospitalization, and 69.0% (95% CI, 18.6–88.2) for the prevention of Covid-19-related ICU admission (Table 1). We did not estimate vaccine effectiveness against fatal outcomes because only two deaths were observed in the unvaccinated group on February 26, 2022.

Table 1
Effectiveness of the CoronaVac Covid-19 vaccine in preventing Covid-19 outcomes among children 3–5 years of age in the study cohort according to immunization status, December 6, 2021, through February 26, 2022*

Immunization status		Cases		Vaccine effectiveness (95% CI)	
	Person-days	No.	Incidence rate	Weighted, standard	Weighted, stratified
			1000 person-days	adjustment †	analysis †
Covid-19					
Unvaccinated	29,404,535	7,555	0.2569	–	–
CoronaVac (3–5 year.)	18,499,492	4,562	0.2466	37.9	38.2
(≥ 14 days after 2 dose)				(36.1 ; 39.6)	(36.5 ; 39.9)
Hospitalization					
Unvaccinated	29,579,595	62	0.0021	–	–
CoronaVac (3–5 year.)	18,990,209	23	0.0012	65.2	64.6
(≥ 14 days after 2 dose)				(50.4 ; 75.6)	(49.6 ; 75.2)
Admission to ICU					
Unvaccinated	29,580,825	9	0.0003	–	–
CoronaVac (3–5 year.)	18,993,888	3	0.0002	68.8	69.0
(≥ 14 days after 2 dose)				(18.0 ; 88.1)	(18.6 ; 88.2)

* Covid-19 denotes coronavirus disease 2019, CI denotes confidence intervals, yr. denotes years. We classified participants' status into two categories during the study period: unvaccinated and fully immunized (≥ 14 days after receiving the second dose with CoronaVac). The days between the first dose vaccine administration and the full immunization were excluded from the at-risk person-time. We provide the results for the standard and stratified versions of the Cox hazards model using inverse probability of treatment weighting.

† The analyses were adjusted for age, sex, region of residence, health insurance category (a proxy of household income), nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19 in children, coded as described in Table S1. The standard and stratified versions of the extended Cox proportional-hazards model were fit to test the robustness of the estimates to model assumptions.

Our estimates provide evidence of vaccination effectiveness in children aged 3 to 5 years during the omicron outbreak in Chile. These results are substantially lower than recent preliminary estimates of the effectiveness of two-dose vaccination of CoronaVac in children 6 to 16 years, in a period when B.1.617.2 (Delta) was the predominant circulating SARS-CoV-2 variant.[14] In that study, the estimated effectiveness in children 6 to 16 years was 74.5% (95% CI, 73.8–75.2) for the prevention of Covid-19, 91.0% (95% CI, 87.8–93.4) for the prevention of hospitalization, and 93.8% (95% CI, 87.8–93.4) for the prevention of Covid-19-related ICU admission. The estimates for the subgroup of children aged 6–11 were 75.8% (95% CI, 74.7–76.8) for the prevention of Covid-19 and 77.9% (95% CI, 61.5–87.3) for the prevention of hospitalization.[14] While the estimates are not directly comparable, the lower estimated vaccine effectiveness could be due to omicron or because the cohort included younger children.

Recent research suggests that vaccines may be less effective against omicron. Consistent with our results, an unpublished study in New York,[13] found that the vaccine effectiveness of BNT162b2 for the prevention of Covid-19 and hospitalization decreased from 66–51% and from 85–73% for children aged 12–17 years, respectively. The drop was more considerable among children 5 to 11 years; protection against Covid-19 fell from 68–12%, and protection against hospitalization fell from 100–48%.[13] Results among adults tell the same story. Early data from South Africa reported that BNT162b2 protection against Covid-19 related hospitalization decreased from 93–70% among adults.[15] Among adults in the United Kingdom, two doses of ChAdOx1 nCoV-19 provided no detectable protection against the omicron variant after 20 weeks, and two doses of BNT162b2 were only 8.8% effective against omicron after 25 weeks.[16] The study suggests a BNT162b2 or mRNA-1273 booster substantially increased protection against omicron.[16] Similarly, a study that evaluated serum neutralization against omicron or D614G variant among adult participants with the mRNA-1273 vaccine primary series observed neutralization titers 35 times lower for omicron.[17]

Children's age could also potentially affect vaccine effectiveness estimates for severe disease, as suggested by older children in recent unpublished studies in New York[13] and Chile.[14] Furthermore, clinical trials for Moderna's mRNA-1273 and Pfizer-BioNTech's BNT162b2 in children six months to under five years old are being conducted. Preliminary results for two 3 µg dose schedule, 21 days apart, of the BNT162b2 in children 2 to < 5 years old found disappointing results, although the immune response of children between six months and two years was comparable to that of young adults.[18] Data from the mRNA-1273 vaccine in children have not yet been released.

Observational studies have limitations. Selection bias could affect vaccine effectiveness estimates if the vaccinated and unvaccinated groups are systematically different. We partially addressed this issue by adjusting our estimates with observable confounders that may affect vaccination and the risk of Covid-19. However, we do not have data to assess whether vaccinated and unvaccinated children or their caregivers differ in some unobservable characteristics, such as compliance with Covid-19 protocols. Another limitation in our study relates to genomic surveillance capabilities. The Ministry of Health's

strategy has focused on detecting variants of concern through traveler and community surveillance but uses non-probabilistic sampling (Supplementary Fig.S3, Tables S3 and S4).

To our advantage, data were collected during the omicron outbreak, with the highest transmission rates since the beginning of the pandemic. Vaccination rollout was quick and had high uptake (Supplementary Figure S2). Our estimated vaccine effectiveness reflects a “real-life” situation by including the challenges public health officials face in the field, such as a more diverse set of participants (e.g., with underlying conditions), schedule compliance, logistics, and cold chains. These estimates may be essential for decision making as a complement to controlled clinical trials.

Our results show that the effectiveness of CoronaVac in children 3 to 5 years against Covid-19 during the omicron was modest, although protection against severe disease remained high.

Online Methods

Outcomes

The Ministry of Health in Chile requires that all suspected Covid-19 cases are notified to health authorities through an online platform. Suspected Covid-19 cases require laboratory testing with reverse-transcription polymerase-chain-reaction assay or antigen tests. We estimated the vaccine effectiveness of CoronaVac for children aged 3 to 5 years using three primary outcomes: laboratory-confirmed Covid-19, hospitalization, and admission to the ICU associated with SARS-CoV-2 infection. We considered the time to the onset of symptoms from the beginning of the follow-up, December 6, 2021, as the endpoint of each outcome. We used the onset of symptoms as a proxy for the time of infection. We classified participants status into two categories along the study period: unvaccinated and fully immunized (≥ 14 days after receipt of the second dose with CoronaVac). The period between the first dose administration and 13 days after the second dose was excluded from the at-risk person-time in our analyses.

Model description

To estimate hazard ratios, we used extensions of the Cox hazards model that allowed us to account for the time-varying vaccination status of participants.[19,20] We adjusted for differences in observed individual characteristics by inverse probability of treatment weighting as in marginal structural models,[21] estimating the weights non-parametrically.[22] Vaccine effectiveness was estimated as hazard ratio between the treated and non-treated status. We reported hazard ratios estimates adjusted for age, sex, region of residence, nationality, health insurance category (a proxy of household income), and underlying conditions (Supplementary Tables S1 and S2) under the standard and stratified versions of the Cox hazards model.

Let T_i be the time-to-event of interest, from December 6, 2021, for the i-th individual in the cohort,

$i = 1, ..., n$. Let x_i, $i = 1, ..., n$, be a p-dimensional vector of individual-specific characteristics, such as age and sex, and $z_i(t)$ be the time-dependent treatment indicator. The model assumes that the time-to-events are independent and with probability distribution given by

$$T_i \mid \boldsymbol{x}_i, z_i \overset{ind.}{\sim} f(t \mid \boldsymbol{x}_i, z_i), \quad i = 1, \ldots, n,$$

where

$$\begin{aligned} f(t \mid \boldsymbol{x}_i, z_i) &= \lambda_0(t) \exp\left\{\boldsymbol{x}_i'\gamma + \beta_{z_i(t)}\right\} \times \\ &\quad \exp\left\{-\exp\left\{\boldsymbol{x}_i'\gamma + \beta_{z_i(t)}\right\} \int_0^t \lambda_0(u)du\right\}, \end{aligned}$$

with $\gamma \in \mathrm{R}^p$ being a vector of regression coefficients, $\beta_k \in \mathrm{R}$ being the regression coefficient measuring the effectiveness of the k^{th} vaccine, and λ_0 being the baseline hazard function

$$\lambda_0(t) = \lim_{h \to 0} \left\{ \frac{P_0\left(t \leq T \leq t + h \mid T \geq t\right)}{h} \right\},$$

were P_0 is the baseline probability distribution. A Cox model with time-dependent covariates compares the risk of the event of interest between immunized and non-immunized participants at each event time but re-evaluates which risk group each person belonged to, based on whether they had been immunized by that time.

We also fitted a stratified version of the model,[23] where the time-to-event distribution is given by

$$\begin{aligned} f(t \mid \boldsymbol{x}_i, z_i) &= \lambda_{\boldsymbol{x}_i,0}(t) \exp\left\{\beta_{z_i(t)}\right\} \times \\ &\quad \exp\left\{-\exp\left\{\beta_{z_i(t)}\right\} \int_0^t \lambda_{\boldsymbol{x}_i,0}(u)du\right\}, \end{aligned}$$

with $\beta_k \in \mathrm{R}$ being the regression coefficient measuring the effectiveness of the kth vaccine, and $\lambda_{x,0}$ is the predictor-specific baseline hazard function. We fitted a stratified version of the extended Cox proportional hazards model to test the robustness of our estimates to model assumptions. Under the stratified Cox model, each combination of predictors has a specific hazard function that can evolve independently.

We estimated the vaccine effectiveness as $100\% \bullet \left(1 - exp\{\beta_k\}\right)$. We show the adjusted vaccine effectiveness results, including covariates as controls (age, gender, region, nationality, health insurance category, and comorbidities). We show the results for the standard and stratified versions of the Cox hazards model using inverse probability of treatment weighting. Inference was based on a partial likelihood approach.[24] Please recall that the effectiveness estimate for the Covid-19 vaccines in the Cox model with time-dependent vaccination status compares the risk of an event for children who received the vaccine and those who were unvaccinated at each event time. The risk group is determined by whether the child had received or not the vaccine shot in a specific calendar time, and the comparison of the risk of an event is made at the same calendar time. Each term in the partial likelihood of the effectiveness regression coefficient corresponds to the conditional probability of an individual to express the outcome of interest from the risk set at a given calendar time.

Under the standard Cox model, all individuals at risk are included in the risk set, and their contribution is weighted based on their covariates (as shown in Supplementary Table S1). Under the stratified version of the Cox model, each stratum has a different risk set determined by the covariates.

We conducted the analysis with the survival package[25] of R, version 4.0.5.[26]

Declarations

The research protocol was approved by the Comité Ético Científico Clínica Alemana Universidad del Desarrollo. The study was considered exempt from informed consent, no human health risks were identified. Research analysts belong to the Chilean Ministry of Health; our use of data follows Chilean law 19.628 on personal data protection

References

1. Tracking SARS-CoV-2 variants. WHO, 2022. (Accessed March 2, 2022, at https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/)
2. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 2020;20:P533-P4.
3. Madhi SA, Kwatra G, Myers JE, et al. Population Immunity and Covid-19 Severity with Omicron Variant in South Africa. N Engl J Med 2022.
4. Cloete J, Kruger A, Masha M, et al. Paediatric hospitalisations due to COVID-19 during the first SARS-CoV-2 omicron (B.1.1.529) variant wave in South Africa: a multicentre observational study. Lancet Child Adolesc Health 2022.
5. Altarawneh HN, Chemaitelly H, Hasan MR, et al. Protection against the Omicron Variant from Previous SARS-CoV-2 Infection. N Engl J Med 2022.
6. Hoffmann M, Krüger N, Schulz S, et al. The Omicron variant is highly resistant against antibody-mediated neutralization: Implications for control of the COVID-19 pandemic. Cell 2022;185:447 –

56.e11.

7. Wang L, Berger NA, Kaelber DC, Davis PB, Volkow ND, Xu R. COVID infection severity in children under 5 years old before and after Omicron emergence in the US. medRxiv 2022.
8. Iuliano AD, Brunkard JM, Boehmer TK, et al. Trends in disease severity and health care utilization during the early omicron variant period compared with previous SARS-CoV-2 high transmission periods — United States, December 2020–January 2022. Morb Mortal Wkly Rep 2022;71:146–52.
9. Zimmermann P, Pittet LF, Finn A, Pollard AJ, Curtis N. Should children be vaccinated against COVID-19? Arch Dis Child 2021;107:e1.
10. Coronavirus Vaccine Tracker. The New York Times, 2022. (Accessed March 1, 2022, at https://nyti.ms/3nvAEc4)
11. Walter EB, Talaat KR, Sabharwal C, et al. Evaluation of the BNT162b2 Covid-19 vaccine in children 5 to 11 years of age. N Engl J Med 2022;386:35–46.
12. Olson SM, Newhams MM, Halasa NB, et al. Effectiveness of BNT162b2 Vaccine against Critical Covid-19 in Adolescents. N Engl J Med 2022;386:713–23.
13. Dorabawila V, Hoefer D, Bauer UE, Bassett MT, Lutterloh E, Rosenberg ES. Effectiveness of the BNT162b2 vaccine among children 5–11 and 12–17 years in New York after the Emergence of the Omicron Variant. medRxiv 2022.
14. Jara A, Undurraga EA, Flores JC, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Children and Adolescents: A Large-Scale Observational Study. SSRN Preprint:http://dx.doi.org/10.2139/ssrn.4035405.
15. Collie S, Champion J, Moultrie H, Bekker L-G, Gray G. Effectiveness of BNT162b2 vaccine against omicron variant in South Africa. N Engl J Med 2022;386.
16. Andrews N, Stowe J, Kirsebom F, et al. Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529) Variant. N Engl J Med 2022.
17. Pajon R, Doria-Rose NA, Shen X, et al. SARS-CoV-2 Omicron Variant Neutralization after mRNA-1273 Booster Vaccination. N Engl J Med 2022.
18. Pfizer and BioNTech Provide Update on Ongoing Studies of COVID-19 Vaccine. 2022. (Accessed March 1, 2022, at https://bit.ly/3J3GZHc)
19. Jara A, Undurraga EA, González C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. N Engl J Med 2021;385:875–84.
20. Cox DR. Regression models and life-tables. J R Stat Soc Series B Stat Methodol 1972;34:187–202.
21. Robins JM, Hernan MA, Brumback B. Marginal structural models and causal inference in epidemiology. Epidemiology 2000;11:550–60.
22. Cole SR, Hernán MA. Constructing inverse probability weights for marginal structural models. Am J Epidemiol 2008;168:656–64.
23. Therneau TM, Grambsch PM. Modeling Survival Data: Extending the Cox Model: Springer; 2000.

24. Thompson MG, Burgess JL, Naleway AL, et al. Prevention and Attenuation of Covid-19 with the BNT162b2 and mRNA-1273 Vaccines. N Engl J Med 2021;385:320–9.
25. Therneau TM. A Package for Survival Analysis in R. R package version 3.1–12. Computer software] Rochester, MN: Mayo Clinic Retrieved from https://CRAN R-project org/package = survival 2020.
26. R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2019.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- MSCovid35SuppMatNMedv10.docx
- flatAraosepc.pdf
- flatAraosrs.pdf

CoronaVac

O que a ciência comprova

6.7. CoronaVac demonstra mais de 90% de efetividade contra Covid-19 em crianças, mostra amplo estudo chileno

Uma pesquisa conduzida no Chile demonstrou que a efetividade da CoronaVac em crianças e adolescentes de seis a 16 anos alcançou mais de 90% contra hospitalizações e internações relacionadas à Covid-19, reforçando a importância da imunização desse público. Este é o primeiro estudo de efetividade da CoronaVac feito em crianças, ou seja, que avalia a eficácia do imunizante com dados do mundo real. Ele foi conduzido pelo Ministério da Saúde chileno, pela Pontifícia Universidade Católica do Chile e pela Universidade de Harvard, dos Estados Unidos, entre outras instituições, e foi publicado na plataforma de preprints SSRN da The Lancet.

"Esse tipo de estudo é essencial, pois reflete os desafios reais de uma campanha de vacinação, como logística e calendário vacinal, e inclui uma população mais diversificada do que os ensaios clínicos controlados", apontam os autores.

Os cientistas incluíram na pesquisa dois milhões de crianças e adolescentes de seis a 16 anos divididos em dois grupos: imunizados com duas doses de CoronaVac e não vacinados. No grupo coorte total, foram observadas 12.735 infecções por Covid-19, 207 hospitalizações e 30 internações em UTI associadas ao coronavírus.

Resultados

Nas crianças e adolescentes entre seis e 16 anos que tomaram a vacina, a efetividade da CoronaVac foi de 74,5% para prevenir a infecção, 91% contra hospitalizações e 93,8% para evitar internação em Unidade de Terapia Intensiva (UTI). A efetividade do imunizante contra mortes não foi estimada, pois não foi reportado nenhum óbito nessa faixa etária durante o período do estudo.

Já em um subgrupo de crianças de seis a 11 anos, a efetividade foi de 75,8% contra a doença e 77,9% para prevenir hospitalizações. Nenhuma criança vacinada foi internada na UTI, mas seis crianças que não tomaram o imunizante precisaram de internação. Os números mais baixos em relação ao grupo 6-16 podem ser explicados pelas poucas hospitalizações nesse público durante o período do estudo.

Nas análises, os pesquisadores consideraram características socioeconômicas e demográficas e incluíram indivíduos com comorbidades, como doença renal crônica, diabetes, câncer, doenças cardiovasculares, HIV, epilepsia, hemofilia, asma, fibrose cística, artrite idiopática juvenil e lúpus.

"Nossos resultados são consistentes comparados ao estudo de efetividade da CoronaVac em indivíduos chilenos maiores de 16 anos, publicado anteriormente em 2021, que atestou eficácia de 95% na prevenção de infecções, 87,5% contra hospitalizações e 90,3% para internações na UTI em adultos", afirmam os cientistas no artigo.

Vacinação salva a vida de crianças e freia a transmissão do coronavírus

Os pesquisadores chilenos chamam a atenção para os diversos benefícios da imunização de crianças, como prevenir casos graves e mortes nessa população e evitar outras complicações da Covid-19, como a Síndrome Inflamatória Multissistêmica Pediátrica (SIM-P), uma resposta inflamatória severa do organismo que pode levar à morte.

"A vacinação também pode reduzir a transmissão do SARS-CoV-2 para outras crianças e adultos, o que futuramente pode diminuir a necessidade de intervenções não farmacológicas, como quarentena e fechamento de escolas. Essas intervenções já afetaram a educação e a saúde mental das crianças e têm aumentado as desigualdades", ressaltam.

Publicado em: 15/02/2022

Effectiveness of an inactivated SARS-CoV-2 vaccine in children and adolescents: A large-scale observational study

Alejandro Jara Ph.D.[1,2,3], Eduardo A. Undurraga Ph.D.[4,5,6,7], Juan Carlos Flores M.D.[8], José R. Zubizarreta Ph.D.[9,10,11], Cecilia González M.D.[1], Alejandra Pizarro M.D.[1,8], Duniel Ortuño-Borroto D.D.S.[1], Johanna Acevedo M.S.[1], Katherinne Leo B.S.E.[1], Fabio Paredes M.Sc.[1], Tomás Bralic M.S.[1], Verónica Vergara M.S.[1], Francisco Leon M.B.A.[1], Ignacio Parot M.B.A.[1], Paulina Leighton B.S.E.[1], Pamela Suárez B.S.E[1], Juan Carlos Rios Ph.D.[1,8], Heriberto García-Escorza M.S.[1], and Rafael Araos, M.D.[1,5,12,13]*

[1] *Ministry of Health, Santiago, Chile*

[2] *Facultad de Matemáticas, Pontificia Universidad Católica de Chile, Santiago, Chile*

[3] *Center for the Discovery of Structures in Complex Data (MiDaS), Santiago, Chile*

[4] *Escuela de Gobierno, Pontificia Universidad Católica de Chile, Santiago, RM, Chile*

[5] *Initiative for Collaborative Research in Bacterial Resistance (MICROB-R), Santiago, Chile*

[6] *Research Center for Integrated Disaster Risk Management (CIGIDEN), Santiago, Chile*

[7] *CIFAR Azrieli Global Scholars program, CIFAR, Toronto, Canada*

[8] *Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile*

[9] *Department of Health Care Policy, Harvard Medical School, Boston, Massachusetts, USA*

[10] *Department of Biostatistics, Harvard T.H. School of Public Health, Boston, Massachusetts, USA*

[11] *Department of Statistics, Harvard T.H. School of Public Health, Boston, Massachusetts, USA*

[12] *Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina, Universidad del Desarrollo, Santiago, Chile*

[13] *Advanced Center for Chronic Diseases (ACCDiS), Santiago, Chile*

*__Correspondence to__ Dr. Rafael Araos at Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina Clínica Alemana Universidad del Desarrollo, Av. Las Condes 12461, Las Condes, Región Metropolitana, Chile. rafaelaraos@udd.cl

Abstract

Background. Policymakers urgently need evidence to adequately balance the costs and benefits of mass vaccination against Covid-19 across all age groups, including children and adolescents.

Methods. We used a large prospective national cohort of about two million children and adolescents 6 to 16 years to estimate the effectiveness of an inactivated SARS-CoV-2 vaccine (CoronaVac) in preventing Covid-19 cases, hospitalizations, and admission to intensive care unit (ICU). We compared the risk of individuals treated with a complete primary immunization schedule (two doses, 28 days apart) with the risk of unvaccinated individuals during the follow-up period. The study was conducted in Chile from June 27, 2021, to January 12, 2022. We used inverse probability-weighted survival regression models to estimate hazard ratios of complete immunization over the unvaccinated status, accounting for time-varying vaccination exposure and adjusting for relevant demographic, socioeconomic, and clinical confounders.

Findings. The estimated adjusted vaccine effectiveness for the inactivated SARS-CoV-2 vaccine in children aged 6 to 16 years was 74·5% (95% CI, 73·8–75·2), 91·0% (95% CI, 87·8–93·4), 93·8% (95% CI, 87·8–93·4) for the prevention of Covid-19, hospitalization, and ICU admission, respectively. For the subgroup of children 6-11 years, the vaccine effectiveness was 75·8% (95% CI, 74·7–76·8) for the prevention of Covid-19 and 77·9% (95% CI, 61·5–87·3) for the prevention of hospitalization.

Interpretation. Our results suggest that a complete primary immunization schedule with the inactivated SARS-CoV-2 vaccine provides an effective protection against severe Covid-19 disease for children 6-16 years.

Funding. Agencia Nacional de Investigación y Desarrollo (ANID) Millennium Science Initiative Program and Fondo de Financiamiento de Centros de Investigación en Áreas Prioritarias (FONDAP).

Keywords: SARS-CoV-2, Covid-19, vaccine effectiveness, inactivated SARS-CoV-2 vaccine, mRNA vaccine, pediatric cohort

Word count. Text: 3302, Abstract: 243, Tables and Figures: 4, References: 37

Research in context

Evidence before this study

We identified research articles through searches in PubMed and medRxiv, without language restrictions, using the terms ("SARS-CoV-2" OR "Covid-19" OR "2019-nCoV" OR "coronavirus") AND ("vaccine" OR "vaccination") AND ("infant" OR "newborn" OR "child" OR "adolescent"). We searched for studies published between December 1, 2020, and December 31, 2021. We also identified relevant research through the United States National Library of Medicine's website ClinicalTrials.gov. We identified at least seven ongoing phase three clinical trials for children 5-11 years; however, evidence about the efficacy and safety of Covid-19 in pediatric populations is limited, and most studies relate to mRNA vaccines. One study reported preliminary safety and efficacy results from Pfizer-BioNTech's mRNA vaccine BNT162b2's phase 1 and phase 2-3 randomized trial in children 5-11 years, estimating a vaccine efficacy against Covid-19 of 90·7% (95% CI 67·7 to 98·3%) in the United States and Europe. Two articles by the same authors estimated the vaccine effectiveness of BNT162b2 against severe Covid-19 in adolescents 12-18 years in pediatric hospitals in the United States. The first article reported interim findings from 19 hospitals and estimated a vaccine effectiveness against hospitalization of 93% (95% CI 83% to 97%). The second article, including 445 case patients and 777 controls in 31 hospitals, estimated a vaccine effectiveness of 94% (95%CI 90 to 96) against hospitalization and 98% (95%CI 93 to 99) against ICU admission. These studies did not adjust for comorbidities or socioeconomic status. Another study reported preliminary safety findings on vaccine safety collected through passive surveillance during the administration of eight million doses of BNT162b2 in children 5-11 in the United States. Last, two studies assessed the safety and immunogenicity of inactivated SARS-CoV-2 vaccines, Sinovac's CoronaVac and Sinopharm's BBIBP-CorV, in phase 1-2 clinical trials in children and adolescents aged 3-17 years in China. We found no studies examining the efficacy or effectiveness of an inactivated SARS-CoV-2 vaccine in pediatric populations, even though these vaccines account for about half the Covid-19 vaccines doses delivered globally, primarily in low and middle-income countries.

Added value of this study

Our study estimates the effectiveness of the CoronaVac vaccine in preventing Covid-19 cases, hospitalizations, and admission to the intensive care unit (ICU), for children and adolescents aged 6-16. Our estimates are based on a large administrative prospective national cohort of about 2 million children and adolescents to assess the effectiveness of administering a two-dose schedule, adjusting for known demographic, socioeconomic, and clinical confounders of the association between Covid-19 vaccines and outcomes. Vaccine effectiveness estimates are essential, as they reflect real-world challenges of vaccination rollout, such as logistics, cold chains, vaccination schedules, and include more diverse populations than participants in a controlled trial.

Implications of all the available evidence

Our vaccine effectiveness estimates for CoronaVac suggest that a complete primary immunization schedule (two doses, 28 days apart) effectively protects against severe Covid-19 disease for children and adolescents 6-16 years, a finding consistent with the results from phase 2 clinical trials of the vaccine.

Background

The global pandemic of coronavirus disease 2019 (Covid-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has imposed an enormous burden of disease globally. As of January 12, 2022, more than 315 million cases and about 5·5 million deaths have been reported worldwide.[1] Several effective Covid-19 vaccines have been developed and approved since the beginning of the pandemic, and mass vaccination campaigns are now occurring in most countries.[2]

Children and adolescents can develop Covid-19, including severe illness and death. Nevertheless, the risk of severe Covid-19 in healthy children and adolescents under 18 is substantially lower than in adults and typically does not result in medical intervention.[3-6] The most common Covid-19 clinical features in this group include fever, upper respiratory symptoms, and gastrointestinal symptoms, such as diarrhea and vomiting.[7,8] A potentially life-threatening clinical presentation of Covid-19 is the multisystemic inflammatory syndrome (MIS-C). MIS-C's clinical presentation is similar to other hyperinflammatory diseases of children, such as Kawasaki disease, presenting most often with fever and elevated inflammatory markers.[9,10] MIS-C can affect multiple organ systems, including gastrointestinal, mucocutaneous, cardiovascular, and respiratory,[10,11] affecting recovery.[12,13] While MIS-C associated mortality is relatively low (~2%), most patients are admitted to the intensive care unit (ICU); about 40% require inotropic support, and about 15% require mechanical ventilation.[14] Another clinical presentation of concern is long Covid, i.e., persisting symptoms following SARS-CoV-2 infection,[15,16] although data on children and adolescents are still limited. A systematic review suggests that, compared to high-income countries, low and middle-income countries may have a higher burden of pediatric Covid-19 mortality.[17] As seen in adults, comorbidities are associated with a more severe clinical presentation of Covid-19.[18,19]

There are at least seven ongoing clinical trials for Covid-19 vaccines in children 5 to 11 years of age in phase 3.[20] Nevertheless, evidence is scarce on the efficacy and safety of Covid-19 vaccines in pediatric populations,[21-24] and most available evidence relates to mRNA vaccines. Two studies assessed the safety

and immunogenicity of inactivated SARS-CoV-2 vaccines, Sinovac's CoronaVac and Sinopharm's BBIBP-CorV, in phase 1-2 clinical trials in children and adolescents aged 3-17 years in China.[23,24] Only one study of real-life vaccine effectiveness in adolescents 12-18 years for Pfizer–BioNTech's BNT162b2 mRNA Covid-19 vaccine is available.[25,26] We found no articles examining the efficacy or effectiveness of an inactivated SARS-CoV-2 vaccine in pediatric populations, although these vaccines account for about half the Covid-19 vaccines doses delivered globally.[27] Vaccine effectiveness estimates are essential, as they reflect real-world challenges of vaccination rollout, such as logistics, cold chains, vaccination schedules, and include more diverse populations than participants in a controlled trial. Policymakers urgently need evidence to adequately balance the costs and benefits of mass vaccination across all age groups.[28]

Several regulatory agencies have granted emergency authorization to vaccinate children, including the US Food and Drug Administration and the European Medicines Agency, and numerous countries have begun vaccinating children.[2] On June 27, 2021, Chile began vaccinating children and adolescents, following an age-based publicly available schedule. Based on emergency use approvals by the Public Health Institute of Chile, children aged 6-11 received a two-dose schedule of CoronaVac, and children 12-16 years received two doses of CoronaVac or BNT162b2. Doses were administered 28 days apart for both vaccines. As described elsewhere, a national immunization registry keeps track of the vaccination schedules.[29]

Using a large administrative observational dataset of about two million children and adolescents, we estimated the effectiveness of the CoronaVac vaccine in preventing Covid-19 cases, hospitalizations, and admission to the intensive care unit (ICU), for individuals aged 6-16. We also provide vaccine effectiveness estimates among children 6-11 years. We estimated the effectiveness of administering a two-dose schedule, adjusting for relevant demographic, socioeconomic, and pediatric clinical confounders of the association between Covid-19 vaccines and the outcomes. We expect these results to inform policymakers, public health officials, and funders considering Covid-19 vaccination for children.

Methods

Study population and design

Our study is based on a prospective pediatric observational cohort at the national level in Chile. The cohort includes children and adolescents 6 to 16 years of age, followed between June 27, 2021, and January 12, 2022. The anonymity of all participants was preserved during all stages of the study. We included all children and adolescents 6 to 16 years of age affiliated with the national public health insurance program (FONASA, Fondo Nacional de Salud). About 80% of the Chilean population are affiliated with FONASA. Children or adolescents with probable or confirmed SARS-CoV-2 infection by reverse-transcription polymerase-chain-reaction (RT-PCR) or antigen test before June 27, 2021, were excluded from the study. We also excluded children who received any Covid-19 vaccine before June 27, 2021. For children that received a vaccine booster (third dose) during the study period, the follow-up was stopped at the date of the booster administration.

The Public Health Institute of Chile, the regulatory authority responsible for pharmacovigilance in Chile, approved the BNT162b2 Covid-19 vaccine for adolescents 12-16 years of age on May 31, 2021. The use of CoronaVac for children aged six years and older was authorized on September 6, 2021. By program indication, children aged 6-11 received CoronaVac, and children 12-16 received CoronaVac or BNT162b2. Both vaccines were administered in two doses, 28 days apart. We did not focus on the effectiveness of the BNT162b2 vaccine, because those results have been provided elsewhere.[25,26] Nevertheless, we provide estimates of the effectiveness of the BNT162b2 in the Supplementary material as a robustness check to our methods. We focused on the effectiveness of the CoronaVac vaccine in children as those results are not available and CoronaVac is among the most used vaccine globally.[27]

We classified participants into two groups: fully immunized, defined as those with a complete vaccination schedule starting 14 days after receiving the second dose, and unvaccinated individuals. The national

vaccination campaign in Chile is described in more detail in the Supplementary material and previous publications.[29,30]

The study team was entirely responsible for the study design, data collection, and analysis. The authors vouch for the accuracy and completeness of the data. The first, second, third, and last authors wrote the first draft of the manuscript.

Outcomes and covariates

We estimated the vaccine effectiveness of CoronaVac for children aged 6-16 using three primary outcomes: laboratory-confirmed Covid-19, hospitalization, and admission to the ICU associated with SARS-CoV-2 infection. We also provide estimates of the vaccine effectiveness of CoronaVac for the prevention of Covid-19 and hospitalization in the subgroup of children aged 6-11. We did not estimate vaccine effectiveness against fatal outcomes because no deaths have been observed in the cohort as of January 12, 2022. The time to the onset of symptoms from the beginning of the follow-up, June 27, 2021, was considered as the endpoint of each outcome. In Chile, all suspected Covid-19 cases are notified to health authorities using an online platform and confirmed by laboratory testing, by reverse polymerase chain reaction (RT-PCR), and antigen test for SARS-CoV-2.

We considered relevant demographic, socioeconomic, and clinical confounders of the association between Covid-19 vaccines and the outcomes of interest. The covariates included age, sex, region of residence, health insurance category (a proxy of household income), nationality, and whether the individual had underlying conditions that has been associated with severe Covid-19 illness in children. These conditions included end-stage chronic kidney disease, diabetes mellitus types 1 and 2, cancer, congenital heart disease, human immunodeficiency virus (HIV) infection, epilepsy, hemophilia, asthma, cystic fibrosis, juvenile idiopathic arthritis, and systemic lupus erythematosus.

Statistical analysis

We determined vaccine effectiveness by estimating the hazard ratio between the treated (complete vaccination schedule) and non-treated unvaccinated status, using the observed time-to-onset of symptoms, from June 27, 2021, through January 12, 2022. We estimated hazard ratios based on an extended version of the Cox hazards model to allow for the time-varying vaccination status of children in the cohort.[29,31] We adjusted for differences in observed individual characteristics by inverse probability of treatment weighting as in marginal structural models,[32] estimating the weights non-parametrically based on observed characteristics.[33] We present the hazard ratio estimates using the standard and stratified versions of the Cox hazards model (please see the supplementary material for more details), adjusting by individual's age, sex, region of residence, nationality, health insurance category, and underlying health conditions, to show that our results do not hinge on model specification. Vaccine effectiveness was defined as one minus the corresponding hazard ratio. The comparison of the risk of an event for fully vaccinated and unvaccinated children is made at the same calendar time. Each term in the partial likelihood of the effectiveness regression coefficient corresponds to the conditional probability of an individual to express the outcome of interest from the risk set at a given calendar time. The inference was based on a partial likelihood approach. Statistical analyses were conducted using the survival package of R version 4.0.5.

Role of the funding source

The funders of this study had no role in the study design, in the collection, analysis, and interpretation of data, in the writing of this manuscript or in the decision to submit the paper for publication.

Findings

Study population

Figure 1 shows the flow diagram of the study cohort. The cohort included 2,086,108 children and adolescents between six and 16 years of age affiliated to FONASA. Of these, 1,976,344 were included in

the study as they did not have a Covid-19 history or had been vaccinated against Covid-19 before June 27, 2021. The descriptive statistics for the study cohort are presented in Table 1. Additional descriptive statistics, including the region of residence and underlying conditions, are provided in tables S1 and S2 (Supplementary material). All variables showed statistically significant differences in the incidence of Covid-19 and for vaccination status.

Vaccine effectiveness

The total follow-up period included approximately 120 million person-days in the CoronaVac group (children 6-16 years) and 230 million person-days in the unvaccinated group (Table 2). The overall cohort had 12,735 events of Covid-19 disease, 207 hospitalizations, and 30 ICU admissions associated with SARS-CoV-2 confirmed infection.

The estimated adjusted vaccine effectiveness for CoronaVac in children aged 6 to 16 years, with a complete primary immunization was 74·5% (95% CI, 73·8–75·2) for the prevention of Covid-19, 91·0% (95% CI, 87·8–93·4) for the prevention of hospitalization, and 93·8% (95% CI, 87·8–93·4) for the prevention of Covid-19-related ICU admission (Table 2). For the subgroup of children 6-11 years, the estimated adjusted vaccine effectiveness for CoronaVac with a complete primary immunization was 75·8% (95% CI, 74·7–76·8) for the prevention of Covid-19 and 77·9% (95% CI, 61·5–87·3) for the prevention of hospitalization. There were only six children 6-11 years admitted to the ICU in the unvaccinated group and none among those who received CoronaVac (Table 3). This results in an estimated 100% vaccine effectiveness for the prevention of Covid-19-related ICU admission, but more data would most likely result in a lower estimate.

Last, the estimated adjusted vaccine effectiveness for BNT162b2 in adolescents aged 12 to 16 years, with a complete primary immunization, was 84·4% (95% CI, 83·7–85·0) for the prevention of Covid-19, 93·5% (95% CI, 90·4–95·6) for the prevention of hospitalization, and 98·0% (95% CI, 89·9–99·6) for the prevention of ICU admission (Table S3, Supplementary material).

Discussion

This study provides estimates of the effectiveness of an inactivated SARS-CoV-2 vaccine (CoronaVac) in children and adolescents 6-16 years of age in a countrywide mass vaccination campaign to prevent laboratory-confirmed Covid-19, hospitalization and Covid-19-related ICU admission. For children and adolescents with a complete primary immunization with CoronaVac, the adjusted vaccine effectiveness was 74·5%, 91·0%, and 93·8% for Covid-19, hospitalization, and ICU admission. The subgroup of children 6-11 years had an adjusted vaccine effectiveness of 75·8% for the prevention of Covid-19 and 77·9% for the prevention of hospitalization.

While there are no publicly available estimates of CoronaVac's effectiveness in children and adolescents, our results are consistent with estimates of the effectiveness of the CoronaVac vaccine in preventing Covid-19 in an adult cohort 16 years and older in Chile in early 2021.[29] The study found and adjusted vaccine effectiveness of 65·9% (95% CI, 65·2-66·6) for the prevention of Covid-19, 87·5% (95% CI, 86·7 to 88·2) for the prevention of hospitalization, and 90·3% (95% CI, 89·1 to 91·4) for the prevention of ICU admission in adults. For children 6-11 years with a complete primary immunization with CoronaVac, the adjusted vaccine effectiveness was 75·8% for preventing Covid-19 and 77·9% for hospitalization. The low baseline risk for presenting severe disease among unvaccinated children and few hospitalization events during the study period may explain the lower effectiveness estimated for this group. Similar to previous vaccine effectiveness estimates for adults,[29] our estimates for children and adolescents 6-16 years also show higher protection against severe disease than against Covid-19. Last, Han et al. reported the safety and immunogenicity of CoronaVac in healthy children and adolescents aged three to 17 years in June 2021. Seroconversion was 100% (98-100) for the 3 μgr dose. Those authors reported no serious events related to the vaccine,[23] consistent with adverse events associated with CoronaVac in Chile (Supplementary material).

As a robustness check to support our approach and analysis, we estimated an adjusted vaccine effectiveness for adolescents with a complete primary immunization using BNT162b2 of 84·4%, 93·5%, and 98·0% for Covid-19, hospitalization, and Covid-19 related ICU admission associated with SARS-CoV-2 infection. Our BNT162b2 vaccine effectiveness estimates for adolescents are consistent to the results of a multicenter case-control study of fully immunized adolescents 12 to 18 years old in June through October 2021 in the United States.[25,26] The study reported vaccine effectiveness of 94% (95%CI 90 to 96) to prevent Covid-19 related hospitalizations and 98% (95%CI 93 to 99) against ICU admission. The study estimated vaccine effectiveness in a period when B.1.617.2 (Delta) was the dominant circulating SARS-CoV-2 variant. Delta was also the predominant variant during the study period in Chile (Supplementary material). Furthermore, a recent study reported a vaccine efficacy against Covid-19 of 90·7% (95% CI 67·7-98·3) for BNT162BT in 5-to-11-year-old children.[22] Our vaccine effectiveness estimate for protection against Covid-19 in 12-to-16-year-old children was a slightly lower, 84·4% (95% CI 83·7–85·0), but within their estimated confidence intervals.

There is an ongoing scientific debate about the convenience of vaccinating children against Covid-19.[28,34] The cost-benefit analysis is not straightforward, particularly when considering global Covid-19 vaccination targets and inequities in vaccine access.[34] Vaccinating children and adolescents against SARS-CoV-2 has several potential benefits.[28] First, it prevents Covid-19 cases, particularly severe illness and potential deaths among children with underlying health conditions. Second, it may prevent long-term consequences of SARS-CoV-2 infection, including MIS-C and long Covid. Third, vaccination may reduce transmission to other children and adults and, by mitigating community transmission, may help reduce the need for non-pharmaceutical interventions such as lockdowns, school exclusions and closures, and quarantines. These interventions have already affected children's educational attainment, mental health, school services, and have increased inequalities.[34-36] There is increasing evidence that vaccinating children and adolescents may significantly reduce the disease burden of Covid-19. Longer follow-up will allow responding whether vaccines can help prevent long-term complications, such as MIS-C and

persistent symptoms following severe SARS-CoV-2 infection, such as headaches, fatigue, and sleep disturbances.[16] We hope our estimates will help inform this ongoing debate and support urgent decision-making globally in responding to Covid-19.

The main strengths of this study include the use of a large cohort of about two million children, aged six to 16 years, combining administrative and healthcare data that represents about 80% of the Chilean population. This large sample size allowed us to non-parametrically estimate the inverse probability of treatment weights and fit a stratified extended Cox proportional hazards model for the different outcomes of interest (each combination of predictors has a specific hazard function), adding robustness to our statistical approach. Our real-world estimates examine one of the most widely used Covid-19 vaccines globally and are an essential complement to efficacy estimates from randomized controlled trials.[37]

The main limitations in our study include potential selection and misclassification biases, as in all observational studies. We adjusted for known and observable demographic, socioeconomic, and clinical confounders that could affect vaccine effectiveness estimates. We cannot completely rule out the existence of a potentially systematic unobservable difference between the treated and unvaccinated children. Misclassification bias is unlikely, as Chile has a centralized electronic immunization and laboratory registry and testing for SARS-CoV-2 infection is free and widely available. A second limitation is that Chile lacks representative genomic surveillance data to estimate the true prevalence of variants of concern (Alfa, Beta, Gamma, Delta, and Omicron) that may affect vaccine effectiveness estimates. Genomic surveillance reports by the Ministry of Health (Supplementary material) suggest that the predominant variant during the study period was Delta, although Omicron became important during the final weeks of the study. We lack data to estimate vaccine effectiveness against specific variants of concern.

Overall, our vaccine effectiveness estimates suggest that a complete primary immunization schedule (two doses, 28 days apart) provides an effective protection against severe Covid-19 disease for children 6-16 years.

Acknowledgments

We thank members of the Chilean Covid-19 Advisory Group Drs. Catterina Ferreccio (Pontificia Universidad Católica de Chile), Ximena Aguilera (Universidad del Desarrollo), Maria T. Valenzuela (Universidad de Los Andes), Pablo Vial (Universidad del Desarrollo), Gonzalo Valdivia (Pontificia Universidad Católica de Chile), Fernando Otaiza (Ministerio de Salud), and Alvaro Erazo (Pontificia Universidad Católica de Chile) for advice on study design and interpretation of results.

Funding

This research was supported by the Agencia Nacional de Investigación y Desarrollo (ANID) Millennium Science Initiative Program MIDAS [NCN17_059] to AJ; Advanced Center for Chronic Diseases (ACCDiS) ANID FONDAP [15130011] to RA; and Research Center for Integrated Disaster Risk Management (CIGIDEN) ANID FONDAP [15110017] to EU.

Declaration of interests

The authors declare no conflicts of interest.

Data sharing statement

Owing to data privacy regulations, this study's individual-level data used in this study cannot be shared (Law N19.628). Aggregate data on vaccination and Covid-19 incidence are publicly available at https://github.com/MinCiencia/Datos-COVID19/.

Ethics statement

The research protocol was approved by the Comité Ético Científico Clínica Alemana Universidad del Desarrollo. The study was considered exempt from informed consent, no human health risks were identified. Research analysts belong to the Chilean Ministry of Health; our use of data follows Chilean law 19.628 on personal data protection.

References

1. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis* 2020; **20**(5): P533-P4.

2. Zimmer C, Corum J, Wee S-L. Coronavirus Vaccine Tracker. 2021. https://nyti.ms/3nvAEc4 (accessed November 15 2021).

3. Smith C, Odd D, Harwood R, et al. Deaths in children and young people in England after SARS-CoV-2 infection during the first pandemic year. *Nat Med* 2021: 1-8.

4. Götzinger F, Santiago-García B, Noguera-Julián A, et al. COVID-19 in children and adolescents in Europe: a multinational, multicentre cohort study. *Lancet Child Adolesc Health* 2020; **4**(9): 653-61.

5. Castagnoli R, Votto M, Licari A, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review. *JAMA pediatrics* 2020; **174**(9): 882-9.

6. Lu X, Zhang L, Du H, et al. SARS-CoV-2 infection in children. *N Engl J Med* 2020; **382**(17): 1663-5.

7. Mansurian M, Ghandi Y, Habibi D, Mehrabi S. COVID-19 infection in children: A systematic review and meta-analysis of clinical features and laboratory findings. *Archives de Pédiatrie* 2021.

8. Viner RM, Ward JL, Hudson LD, et al. Systematic review of reviews of symptoms and signs of COVID-19 in children and adolescents. *Arch Dis Child* 2021; **106**(8): 802-7.

9. Dionne A, Son MBF, Randolph AG. An Update on Multisystem Inflammatory Syndrome in Children Related to SARS-CoV-2. *Pediatr Infect Dis J* 2022; **41**(1): e6.

10. Feldstein LR, Rose EB, Horwitz SM, et al. Multisystem inflammatory syndrome in US children and adolescents. *N Engl J Med* 2020; **383**(4): 334-46.

11. Yasuhara J, Watanabe K, Takagi H, Sumitomo N, Kuno T. COVID-19 and multisystem inflammatory syndrome in children: A systematic review and meta-analysis. *Pediatr Pulmonol* 2021; **56**(5): 837-48.

12. Dufort EM, Koumans EH, Chow EJ, et al. Multisystem inflammatory syndrome in children in New York State. *N Engl J Med* 2020; **383**(4): 347-58.

13. Jiang L, Tang K, Levin M, et al. COVID-19 and multisystem inflammatory syndrome in children and adolescents. *Lancet Infect Dis* 2020.

14. Kaushik A, Gupta S, Sood M, Sharma S, Verma S. A systematic review of multisystem inflammatory syndrome in children associated with SARS-CoV-2 infection. *Pediatr Infect Dis J* 2020; **39**(11): e340-e6.

15. Nalbandian A, Sehgal K, Gupta A, et al. Post-acute COVID-19 syndrome. *Nat Med* 2021; **27**(4): 601-15.

16. Zimmermann P, Pittet LF, Curtis N. How common is long COVID in children and adolescents? *Pediatr Infect Dis J* 2021; **40**(12): e482.

17. Kitano T, Kitano M, Krueger C, et al. The differential impact of pediatric COVID-19 between high-income countries and low- and middle-income countries: A systematic review of fatality and ICU admission in children worldwide. *PLOS One* 2021; **16**(1): e0246326.

18. Tsankov BK, Allaire JM, Irvine MA, et al. Severe COVID-19 Infection and Pediatric Comorbidities: A Systematic Review and Meta-Analysis. *Int J Infect Dis* 2021; **103**: 246-56.

19. Fernandes DM, Oliveira CR, Guerguis S, et al. Severe acute respiratory syndrome coronavirus 2 clinical syndromes and predictors of disease severity in hospitalized children and youth. *J Pediatr* 2021; **230**: 23-31. e10.

20. United States National Library of Medicine. ClinicalTrials.gov. 2022. https://bit.ly/3o6D5ou (accessed January 22 2022).

21. Hause AM, Baggs J, Marquez P, et al. COVID-19 Vaccine Safety in Children Aged 5-11 Years - United States, November 3-December 19, 2021. *Morb Mortal Wkly Rep* 2021; **70**(5152): 1755-60.

22. Walter EB, Talaat KR, Sabharwal C, et al. Evaluation of the BNT162b2 Covid-19 vaccine in children 5 to 11 years of age. *N Engl J Med* 2022; **386**(1): 35-46.

23. Han B, Song Y, Li C, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21**(12): 1645-53.

24. Xia S, Zhang Y, Wang Y, et al. Safety and immunogenicity of an inactivated COVID-19 vaccine, BBIBP-CorV, in people younger than 18 years: a randomised, double-blind, controlled, phase 1/2 trial. *Lancet Infect Dis* 2022; **22**(2): 196-208.

25. Olson SM, Newhams MM, Halasa NB, et al. Effectiveness of Pfizer-BioNTech mRNA Vaccination Against COVID-19 Hospitalization Among Persons Aged 12-18 Years-United States, June-September 2021. *Morb Mortal Wkly Rep* 2021; **70**(42): 1483.

26. Olson SM, Newhams MM, Halasa NB, et al. Effectiveness of BNT162b2 Vaccine against Critical Covid-19 in Adolescents. *N Engl J Med* 2022.

27. Mallapaty S. China's COVID vaccines have been crucial — now immunity is waning. *Nature* 2021: 14 October

28. Zimmermann P, Pittet LF, Finn A, Pollard AJ, Curtis N. Should children be vaccinated against COVID-19? *Arch Dis Child* 2021.

29. Jara A, Undurraga EA, González C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *N Engl J Med* 2021; **385**: 875-84.

30. Jara A, Undurraga EA, Zubizarreta JR, et al. Effectiveness of Homologous and Heterologous Booster Shots for an Inactivated SARS-CoV-2 Vaccine: A Large-Scale Observational Study. *SSRN Preprint* 2022; **https://dx.doi.org/10.2139/ssrn.4005130**.

31. Cox DR. Regression models and life-tables. *Journal of the Royal Statistical Society: Series B (Methodological)* 1972; **34**(2): 187-202.

32. Robins JM, Hernan MA, Brumback B. Marginal structural models and causal inference in epidemiology. *Epidemiology* 2000; **11**(5): 550-60.

33. Cole SR, Hernán MA. Constructing inverse probability weights for marginal structural models. *American journal of epidemiology* 2008; **168**(6): 656-64.

34. World Health Organization. Interim statement on COVID-19 vaccination for children and adolescents. 2021. https://bit.ly/3fZUqLu (accessed January 20 2022).

35. Christakis DA, Van Cleve W, Zimmerman FJ. Estimation of US Children's Educational Attainment and Years of Life Lost Associated With Primary School Closures During the Coronavirus Disease 2019 Pandemic. *JAMA Netw Open* 2020; **3**(11): e2028786-e.

36. Van Lancker W, Parolin Z. COVID-19, school closures, and child poverty: a social crisis in the making. *Lancet Public Health* 2020; **5**(5): e243-e4.

37. Lopalco PL, DeStefano F. The complementary roles of Phase 3 trials and post-licensure surveillance in the evaluation of new vaccines. *Vaccine* 2015; **33**(13): 1541-8.

Table 1. Characteristics of the study cohort of children and adolescents affiliated to FONASA, overall, with laboratory-confirmed Covid-19, and the proportion receiving one or more doses of Covid-19 vaccines, June 27, 2021 through January 12, 2022*

							Vaccinated					
			COVID-19		Unvaccinated		One dose		Two doses		Three doses	
Characteristic	No.	Col.%	No.	Row%	No.	Row%	No.	Row%	No.	Row%	No.	Row%
Total	1,976,344	100	14,282	0·7	274,042	13·9	138,041	7·0	1,430,124	72·4	134,137	6·8
Sex												
Female	967,074	49·0	7,291	0·75	128,067	13·0	64,903	6·7	703,542	72·7	70,562	7·3
Male	1,009,270	51·0	6,991	0·69	145,975	14·0	73,138	7·2	726,582	72·0	63,575	6·3
Age group												
6	185,179	9·4	992	0·5	43,852	24·0	20,757	11·0	120,569	65·1	1	0·0
7	183,622	9·3	1,025	0·6	36,650	20·0	17,694	9·6	129,277	70·4	1	0·0
8	181,165	9·2	1,138	0·6	32,877	18·0	16,139	8·9	132,148	72·9	1	0·0
9	185,022	9·4	1,256	0·7	30,802	17·0	16,143	8·7	138,077	74·6	0	0·0
10	188,996	9·6	1,428	0·7	28,676	15·0	15,856	8·4	144,464	76·4	0	0·0
11	187,941	9·5	1,514	0·8	23,912	13·0	13,488	7·2	150,260	79·9	281	0·1
12	185,790	9·4	1,489	0·8	19,591	11·0	10,229	5·5	150,447	81·0	5,523	3·0
13	179,140	9·1	1,519	0·8	16,299	9·1	8,752	4·9	147,117	82·0	6,972	3·9
14	173,105	8·8	1,385	0·8	15,146	8·7	7,288	4·2	125,450	72·5	25,221	14·6
15	168,202	8·5	1,266	0·7	13,752	8·2	6,226	3·7	104,537	62·1	43,687	26·0
16	158,182	8·0	1,270	0·8	12,485	7·9	5,469	3·5	87,778	55·5	52,450	33·2
Comorbidities†												
None	1,726,075	87·0	12,146	0·7	244,342	14·0	121,003	7·0	1,244,602	72·1	116,128	6·7
≥ 1	250,269	13·0	2,136	0·8	29,700	12·0	17,038	6·8	185,522	74·1	18,009	7·2
Nationality												
Chilean	1,917,024	97·0	14,044	0·7	260,369	14·0	134,454	7·0	1,391,052	72·6	131,149	6·8
Non-Chilean	59,320	3·0	238	0·4	13,673	23·0	3,587	6·0	39,072	65·9	2,988	5·0

Notes. ***Covid-19 denotes coronavirus disease 2019. The study** cohort included children and adolescents 6-16 years of age affiliated with the Fondo Nacional de Salud (FONASA), the national public health insurance program which collects, manages, and distributes funds for the public healthcare system in Chile. We excluded children or adolescents with probable or confirmed SARS-CoV-2 infection before June 27, 2021, or if they had received any Covid-19 vaccine before June 27, 2021. The model also included health insurance category (a proxy of family income), and location (16 regions). We found statistically significant differences ($p<0{\cdot}001$) between Covid-19 patients and the vaccinated and unvaccinated groups by sex, age group, comorbidities, nationality, region of residence, and category of health insurance. Additional details are shown in Table S1. Covid-19 vaccines include CoronaVac and BNT162b2 (Table 2 and Table S3, respectively).

†Coexisting conditions included chronic kidney disease, diabetes mellitus types 1 and 2, cancer, congenital heart disease, HIV, epilepsy, hemophilia, asthma, cystic fibrosis, juvenile idiopathic arthritis, and systemic lupus erythematosus.

Table 2. Effectiveness of the CoronaVac vaccine in preventing Covid-19 outcomes among children 6-16 years of age in the study cohort according to immunization status, June 27, 2021, through January 12, 2022*

Immunization status		Cases		Vaccine effectiveness (95% CI)	
	Person-days	No.	Incidence rate 1000 person-days	Weighted, standard adjustment †	Weighted, stratified analysis‡
Covid-19					
Unvaccinated	229,123,227	8,648	0·0377	–	–
CoronaVac (6-16 yr.) (≥14 days after 2 dose)	118,833,107	2,998	0·0252	74·8 (74·1–75·5)	74·5 (73·8–75·2)
Hospitalization					
Unvaccinated	229,684,717	181	0·0008	–	–
CoronaVac (6-16 yr.) (≥14 days after 2 dose)	119,666,696	16	0·0001	91·3 (88·1–93·6)	91·0 (87·8–93·4)
Admission to ICU					
Unvaccinated	229,696,288	28	0·0001	–	–
CoronaVac (6-16 yr.) (≥14 days after 2 dose)	119,679,580	1	0·00001	93·8 (85·7–97·3)	93·8 (85·7–97·3)

*****Participants were classified into two groups: those who were unvaccinated and those who were fully immunized (≥14 days after receipt of the second dose) with CoronaVac. The 13 days between vaccine administration and full immunization were excluded from the at-risk person-time. We show the results for the standard and stratified versions of the Cox hazards model using inverse probability of treatment weighting. Covid-19 denotes coronavirus disease 2019, CI denotes confidence intervals.

† The analysis was adjusted for age, sex, 16 regions of residence, health insurance category, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19 in children.

‡ A stratified version of the extended Cox proportional-hazards model was fit to test the robustness of the estimates to model assumptions, stratifying by age, sex, region of residence, health insurance category (a proxy of household income), nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19, and coded as described in Table 1.

Table 3. Effectiveness of the CoronaVac Covid-19 vaccine in preventing Covid-19 outcomes among children 6-11 years of age in the study cohort according to immunization status, June 27, 2021, through January 12, 2022*

Immunization status		Cases		Vaccine effectiveness (95% CI)	
	Person-days	No.	Incidence rate 1000 person-days	Weighted, standard adjustment †	Weighted, stratified analysis‡
Covid-19					
Unvaccinated	155,092,218	5,021	0·0324	–	–
CoronaVac (6-11 yr.)	78,449,194	1,502	0·0191	75·8	75·8
(≥14 days after 2 dose)				(74·8–76·8)	(74·7–76·8)
Hospitalization					
Unvaccinated	155,434,360	61	0·0004	–	–
CoronaVac (6-11 yr.)	78,940,292	8	0·0001	78·5	77·9
(≥14 days after 2 dose)				(62·8–87·6)	(61·5– 87·3)

*****Participants were classified into two groups: those who were unvaccinated and those who were fully immunized (≥14 days after receipt of the second dose) with CoronaVac. The 13 days between vaccine administration and full immunization were excluded from the at-risk person-time. We show the results for the standard and stratified versions of the Cox hazards model using inverse probability of treatment weighting. Covid-19 denotes coronavirus disease 2019, CI denotes confidence intervals. There were only six children 6-11 years admitted to the ICU in the unvaccinated group and none among those who received CoronaVac. This results in an estimated 100.0% vaccine effectiveness for the prevention of Covid-19-related ICU admission, but more data would most likely result in a lower estimate.

† The analysis was adjusted for age, sex, 16 regions of residence, health insurance category, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19 in children.

‡ A stratified version of the extended Cox proportional-hazards model was fit to test the robustness of the estimates to model assumptions, stratifying by age, sex, region of residence, health insurance category (a proxy of household income), nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19, and coded as described in Table 1.

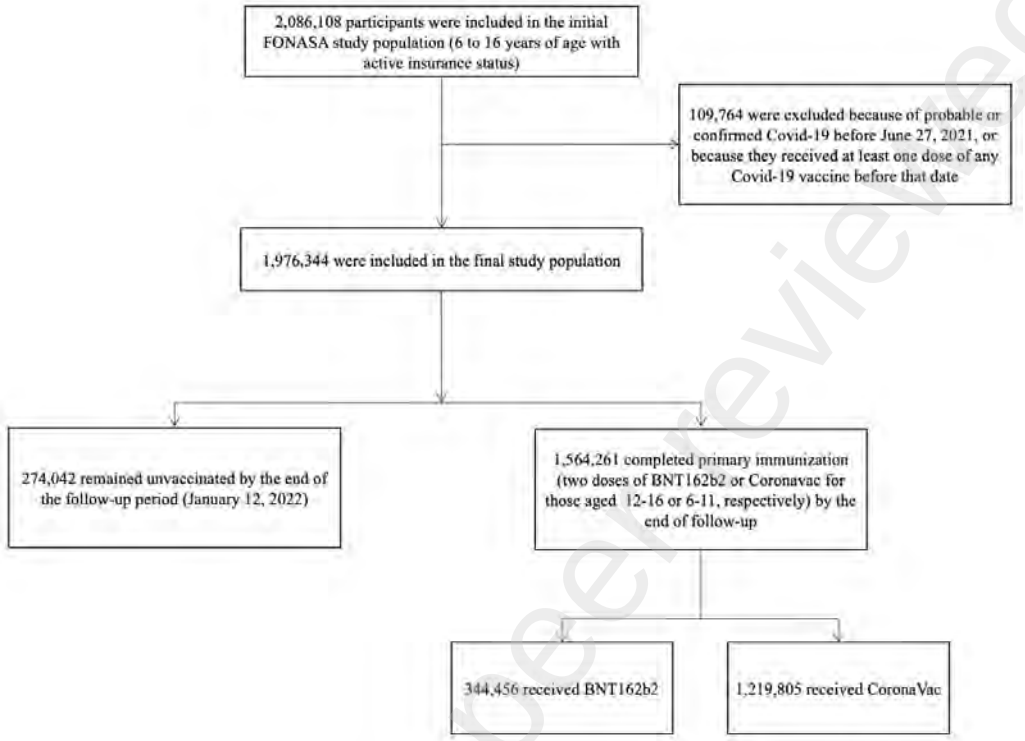


Figure 1. Study participants and cohort eligibility, June 27, 2021, to January 12, 2022. Participants were between 6 to16 years of age, affiliated to the Fondo Nacional de Salud (FONASA), the public national healthcare system, and vaccinated with a complete primary immunization (2 doses 28 days apart) with CoronaVac (6-16 years) or BNT162b2 (12-16 years) Covid-19 vaccines between June 27, 2021, and January 12, 2022, or not receiving any Covid-19 vaccination. We excluded individuals who had probable or confirmed coronavirus disease 2019 (Covid-19) according to reverse-transcription polymerase-chain-reaction assay for SARS-Cov-2 or antigen test before June 27, 2021.

CoronaVac

O que a ciência comprova

6.8. Em surto da delta na China, cerca de 20% dos casos foram em crianças e adolescentes; vacinados com CoronaVac não registraram casos críticos

Em estudo publicado na revista PLOS Neglected Tropical Disease, pesquisadores chineses da Universidade Médica do Sul de Guangzhou (Cantão) e do Centro de Controle e Prevenção de Doenças da província apontaram que um em cada cinco casos do surto da variante delta do vírus SARS-CoV-2 que se abateu sobre a região entre maio e junho de 2021 acometeu menores com idade pré-escolar (1 a 5 anos) e estudantes de 6 a 18 anos. Além disso, dos 153 casos de Covid-19 do surto, cerca de 85% ocorreu entre não vacinados.

Durante o período do estudo, houve sete casos assintomáticos e 146 sintomáticos. Destes, 24 (15,7%) foram considerados leves, 113 (73,9%) moderados, e nove (5,9%) foram considerados críticos. Não houve nenhum caso grave. Dos 153 casos, 116 (84,7%) aconteceram em indivíduos sem cobertura vacinal e 21 (15,3%) em pessoas com esquema de vacinação parcial ou completo da CoronaVac, imunizante do Butantan e da farmacêutica chinesa Sinovac, ou Sinopharm, imunizante chinês que também conta com a tecnologia de vírus inativado. Foram excluídos 16 casos com estado vacinal indeterminado.

"Os sintomas clínicos foram mais leves nos casos com vacinação parcial ou total do que naqueles que não foram vacinados. Notavelmente, nenhum caso crítico foi observado naqueles que foram parcial ou totalmente vacinados, enquanto os nove casos críticos ocorreram todos entre pessoas não vacinadas", ressaltaram os pesquisadores no estudo.

Do total de casos de Covid-19 do surto, 28 (18,3%) foram entre menores de 18 anos, 72 (47,1%) entre pessoas de 19 a 59 anos, 19 (12,4%) na população de 60 a 70 anos e 34 (22,2%) em idosos acima dos 70 anos. Crianças em idade pré-escolar responderam por 3,3% dos casos.

Intensificação da vacinação após surto

Em 21 de maio de 2021, foi relatado o primeiro caso da variante delta em Guangzhou. Em resposta ao ressurgimento da Covid-19 na província, o governo local implementou uma série de medidas de contenção e iniciou a vacinação emergencial de toda a população. No fim de junho, quando o surto acabou, 10,7 milhões dos 15,3 milhões de habitantes haviam sido vacinados com CoronaVac ou Sinopharm (sendo que 8,7 milhões haviam completado o esquema vacinal de duas doses), estendendo a cobertura vacinal para 67% da população da província.

Publicado em: 5/1/2022

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Transmission and containment of the SARS-CoV-2 Delta variant of concern in Guangzhou, China: A population-based study

Li Li[1☯], Zhi-Gang Han[2☯], Peng-Zhe Qin[2☯], Wen-Hui Liu[1,2☯], Zhou Yang[1], Zong-Qiu Chen[2], Ke Li[2], Chao-Jun Xie[2], Yu Ma[2], Hui Wang[2], Yong Huang[2], Shu-Jun Fan[2], Ze-Lin Yan[1], Chun-Quan Ou[1]*, Lei Luo[2]*

1 State Key Laboratory of Organ Failure Research, Department of Biostatistics, Guangdong Provincial Key Laboratory of Tropical Disease Research, School of Public Health, Southern Medical University, Guangzhou, China, **2** Guangzhou Center for Disease Control and Prevention, Guangzhou, China

☯ These authors contributed equally to this work.
* ouchunquan@hotmail.com (C-QO); llyeyq@163.com (LL)

OPEN ACCESS

Citation: Li L, Han Z-G, Qin P-Z, Liu W-H, Yang Z, Chen Z-Q, et al. (2022) Transmission and containment of the SARS-CoV-2 Delta variant of concern in Guangzhou, China: A population-based study. PLoS Negl Trop Dis 16(1): e0010048. https://doi.org/10.1371/journal.pntd.0010048

Editor: Susanna Kar Pui Lau, The University of Hong Kong, HONG KONG

Received: July 22, 2021

Accepted: December 3, 2021

Published: January 5, 2022

Data Availability Statement: The datasets generated and/or analysed during the current study are not publicly available due to the regulations of Guangzhou Center for Disease Control and Prevention. Permission can be requested by contacting Guangzhou Center for Disease Control and Prevention (http://www.gzcdc.org.cn).

Funding: This work was supported by National Natural Science Foundation of China [81973140, 82003555] to CQO and L LI, China Postdoctoral Science Foundation [2020M672744, 2020TQ0135]

Abstract

Background

The first community transmission of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Delta variant of concern (VOC) in Guangzhou, China occurred between May and June 2021. Herein, we describe the epidemiological characteristics of this outbreak and evaluate the implemented containment measures against this outbreak.

Methodology/Principal findings

Guangzhou Center for Disease Control and Prevention provided the data on SARS-CoV-2 infections reported between 21 May and 24 June 2021. We estimated the incubation period distribution by fitting a gamma distribution to the data, while the serial interval distribution was estimated by fitting a normal distribution. The instantaneous effective reproductive number (R_t) was estimated to reflect the transmissibility of SARS-CoV-2. Clinical severity was compared for cases with different vaccination statuses using an ordinal regression model after controlling for age. Of the reported local cases, 7/153 (4.6%) were asymptomatic. The median incubation period was 6.02 (95% confidence interval [CI]: 5.42–6.71) days and the means of serial intervals decreased from 5.19 (95% CI: 4.29–6.11) to 3.78 (95% CI: 2.74–4.81) days. The incubation period increased with age ($P<0.001$). A hierarchical prevention and control strategy against COVID-19 was implemented in Guangzhou, with R_t decreasing from 6.83 (95% credible interval [CrI]: 3.98–10.44) for the 7-day time window ending on 27 May 2021 to below 1 for the time window ending on 8 June and thereafter. Individuals with partial or full vaccination schedules with BBIBP-CorV or CoronaVac accounted for 15.3% of the COVID-19 cases. Clinical symptoms were milder in partially or fully vaccinated cases than in unvaccinated cases (odds ratio [OR] = 0.26 [95% CI: 0.07–0.94]).

to L LI, the Key Project of Medicine Discipline of Guangzhou [2021–2023-11] to L LUO, and the Basic Research Project of Key Laboratory of Guangzhou [202102100001] to L LUO. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Conclusions/Significance

The hierarchical prevention and control strategy against COVID-19 in Guangzhou was timely and effective. Authorised inactivated vaccines are likely to contribute to reducing the probability of developing severe disease. Our findings have important implications for the containment of COVID-19.

Author summary

On 11 May 2021, the WHO reclassified the B.1.617.2 variant as a "variant of concern" (VOC) from being a "variant of interest", considering its global public health significance. On 21 May 2021, the first local case infected with the Delta variant (i.e. lineage B.1.617.2) in Guangzhou, China, was reported. In response to the resurgence of COVID-19, the local government implemented a series of containment measures. This provides a valuable opportunity to understand the characteristics of the Delta variant and to evaluate the performance of inactivated COVID-19 vaccines (BBIBP-CorV and CoronaVac) and other interventions. We estimated that the median incubation period was 6.02 days and the means of serial intervals decreased from 5.19 to 3.78 days. The incubation period increased with age. The vaccination coverage in the COVID-19 cases was 15.3%. Clinical symptoms were milder in cases with partial or full vaccination than in those who were unvaccinated (odds ratio [*OR*] = 0.26). We found that the effective reproductive number decreased from 6.83 for the 7-day time window ending on 27 May 2021 to below 1 for the time window ending on 8 June and thereafter. Our findings have important implications for the containment of COVID-19.

Introduction

Coronavirus disease 2019 (COVID-19) is a serious threat to public health. Globally, there have been over 186 million confirmed cases and 4.0 million deaths as of 11 July 2021 [1], and many efforts, such as non-pharmaceutical interventions (NPIs) and vaccination, have been implemented to prevent and contain COVID-19. The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants has accelerated the spread of COVID-19 [2]. In 2021, explosive surges of SARS-CoV-2 occurred in India. Circulation of the Delta variant (i.e. lineage B.1.617.2), which was first identified in India, may have contributed to the devastating second wave of COVID-19 in India [3]. On 11 May 2021, the WHO reclassified the B.1.617.2 variant as a "variant of concern" (VOC) from being a "variant of interest", considering its global public health significance [4]. The variant has invaded more than 110 countries, territories, and areas [1]. Meanwhile, this variant accounts for a large proportion of the newly sequenced and genotyped SARS-CoV-2 cases in some locations, such as England (>90%) [5]. Understanding the epidemiological characteristics and clinical severity of the SARS-CoV-2 Delta variant would help inform targeted interventions for containing the spread of COVID-19.

Population movement is a critical influential factor of COVID-19 transmission [6]. Guangzhou is an important transportation hub in southern China, with over 15 million permanent residents and mass population mobility. In the first five months of 2021, around 2,000 passengers were arriving in Guangzhou from abroad each day. The city is at high risk for COVID-19

transmission from imported cases from abroad [7]. There were, on average, eight COVID-19 cases imported from abroad every day and no local case was reported between 1 January and 20 May 2021. On 21 May, a local case infected with the Delta variant was reported in Guangzhou [8]. In response to the resurgence of COVID-19, the local government implemented a series of containment measures, including vaccination programs, case finding through mass tests for COVID-19, case isolation, as well as other social distancing interventions. Timely assessment of the epidemiological features of the cases of SARS-CoV-2 infection and the prevention and control measures would provide better preparedness for the COVID-19 outbreak caused by highly infectious variants [9].

Several studies have reported promising vaccine efficacy results based on data collected from clinical trials. More real-world data are needed to elucidate vaccine effectiveness [10]. As of 31 May, over 10 million residents (vaccination coverage: around 67%) in Guangzhou had received COVID-19 vaccines (BBIBP-CorV or CoronaVac), among whom, more than three million residents had been fully vaccinated [11]. This provides a valuable opportunity to evaluate the performance of the authorised inactivated COVID-19 vaccines. Herein, we describe the epidemiological characteristics of the cases infected with SARS-CoV-2 Delta VOC in Guangzhou and evaluate the implemented containment measures.

Methods

Ethics statement

This study was approved by the Research Ethics Committee of Guangzhou CDC (No: GZCDC-ECHR-2020P0019). Consent to participate was waived since anonymous information was used.

Data collection

The Guangzhou Center for Disease Control and Prevention (CDC) provided the individual data of all SARS-CoV-2 infections reported between 21 May and 24 June 2021 in Guangzhou. Nasal and throat swabs were collected for COVID-19 tests. Cases were confirmed to be SARS--CoV-2 infections using real-time reverse transcription-polymerase chain reaction (rRT-PCR, S1 File). The individual information included sex, age, occupation class (people who have retired and the unemployed, preschool children, students, healthcare workers, others), possible infection date, type of exposure (family, having been at the same restaurant with a confirmed case, others), type of detection (tracing of close contacts, mass screening, hospital screening), date of illness onset (the date of symptom onset for the symptomatic cases and the date of sample collection for the first positive test of asymptomatic cases), clinical severity (asymptomatic, mild, moderate, severe, and critical according to the criteria proposed by the National Health Commission of the People's Republic of China [12], S1 Table).

Seventy-five cases who did not have information on the exact infection date and who did not have symptoms were excluded when estimating the incubation period (i.e. the time delay from infection to symptom onset) distribution in the main analysis. A transmission pair was defined as two confirmed COVID-19 cases that had clear epidemiological links with each other, i.e. one case (infectee) was infected by the other (infector). Asymptomatic infectees and the infectees whose infectors were asymptomatic were excluded when estimating the serial interval (i.e. the delay between symptom onset dates of successive cases in transmission pairs) distribution. Symptom onset dates of 67 transmission pairs were used to estimate the serial interval distribution (S1 Fig).

Statistical analysis

The median and range were calculated for the continuous variable of age, and proportions were provided for categorical variables. We estimated the incubation period distribution by fitting a lognormal, gamma, and Weibull distribution to the data using the maximum likelihood method and selected the distribution with the smallest value of Akaike Information Criteria (AIC). The serial interval distributions were estimated by fitting normal distributions [13,14]. We estimated the distributions of serial intervals for the entire study period and for nine different time windows (i.e. eight running time windows with a fixed length of 14 days and the last one was from 26 May through 24 June, making sure that all of the time windows contained at least 30 data points of serial intervals). We assessed the association between age and incubation period using a gamma regression model with a log link (according to the selected distribution for incubation period), while the associations between age (of infector and infectee) and serial interval were examined in linear regression models, after controlling for the effects of calendar time.

Previous studies have suggested that the instantaneous reproductive number is a better choice to examine the effectiveness of control measures compared with the case reproductive number [15]. In this study, we estimated the instantaneous effective reproductive number R_t (the average number of secondary cases arising from a typical primary infection [16]) to reflect the transmissibility of SARS-CoV-2 and to evaluate the performance of interventions implemented during this outbreak. The R_t was estimated as:

$$R_t = \frac{I_t}{\sum_{s=1}^{t} I_{t-s} w_s}$$

where I_t was the number of incident cases at time t and w_s was estimated with the time-varying distributions of serial intervals [17]. When the time step of data is small, the estimates of R_t can be highly variable and it would be difficult to interpret the results. To deal with this problem, we estimated the R_t over a 7-day time window assuming that the R_t remains constant within the same time window. Such estimate reflects the average transmissibility for the time window of one week. We present the R_t for the time window ending on 27 May and thereafter, since the estimates may be unstable at the very beginning of the outbreak with few cases [15].

We categorized the COVID-19 cases into two groups based on their vaccination status (Group 1: unvaccinated; Group 2: partially or fully vaccinated [infection occurred $\geq$21 days after dose 1]; 16 cases with indeterminate vaccination status [infection occurred $<$21 days after dose 1 or the time interval between the infection date and the vaccination date was unclear] were excluded). The differences in the clinical severity of the local cases by vaccination status were evaluated using an ordinal logistic regression model after controlling for the potentially confounding effect of age.

Sensitivity analysis was conducted to check the robustness of (1) the estimate of incubation period distribution (1a) assuming that the incubation period followed the distributions which were not corresponding to the smallest AIC; (1b) including seven additional cases with the information of possible exposure dates or exposure windows; (2) the association between age and incubation period using the models with three independent variables of age, calendar time, and one potentially influential factor (i.e. occupation, type of exposure or clinical severity) which was statistically significant in bivariate regression models (with calendar time and one potentially influential factor as the independent variables). All analyses were conducted using R software (version 4.1.0; R Foundation for Statistical Computing).

Results

On 18 May 2021, a 75-year-old woman (Case #1) showed symptoms and sought professional help in a hospital. Later, on 21 May, the woman was confirmed to be infected with the Delta VOC. She was the first local case infected with this variant in Guangzhou (Fig 1). SARS-CoV-2 was transmitted from the woman to her friend Case #3 and a waitress (reported outside Guangzhou) when they were having a meal in a restaurant. Her husband was also infected. Case #3 brought SARS-CoV-2 to seven family members and eight friends when having a meal in a restaurant and dancing with friends. Case #19, who infected as many as 16 residents, was

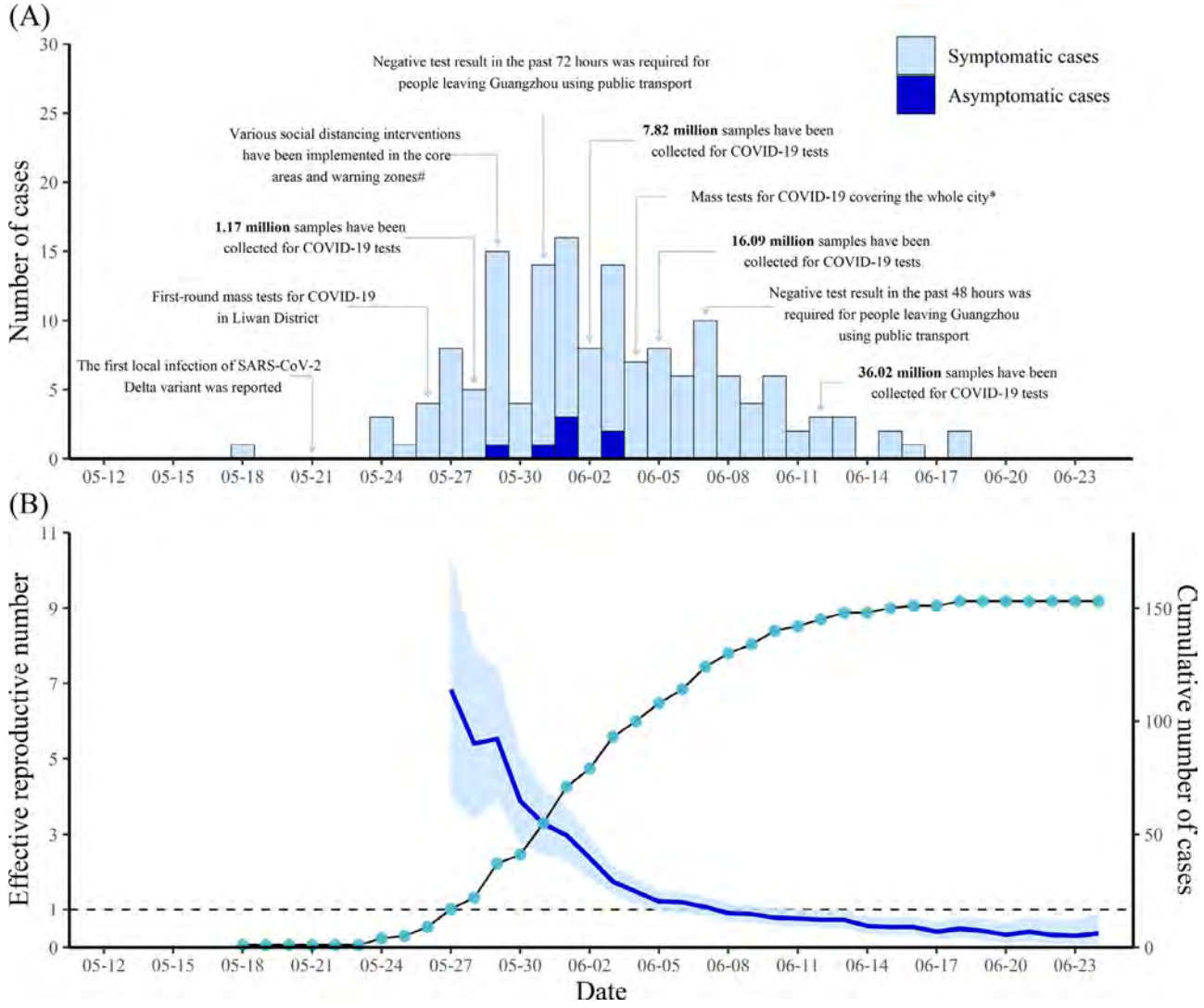


Fig 1. Number of COVID-19 cases by date of illness onset and effective reproductive number in Guangzhou, China. (A) Number of COVID-19 cases by date of illness onset. (B) Estimated effective reproductive number by ending date of 7-day time window and cumulative number of cases by date of illness onset. The blue line shows the point estimates of the effective reproductive number and the light blue region represent the 95% credible intervals. Points represent the daily cumulative number of cases. # Social distancing interventions included school closure, banning of public gatherings, traffic control, prohibition of dining in restaurants. * Mass tests for COVID-19 was done from 4 to 6 June 2021.

https://doi.org/10.1371/journal.pntd.0010048.g001

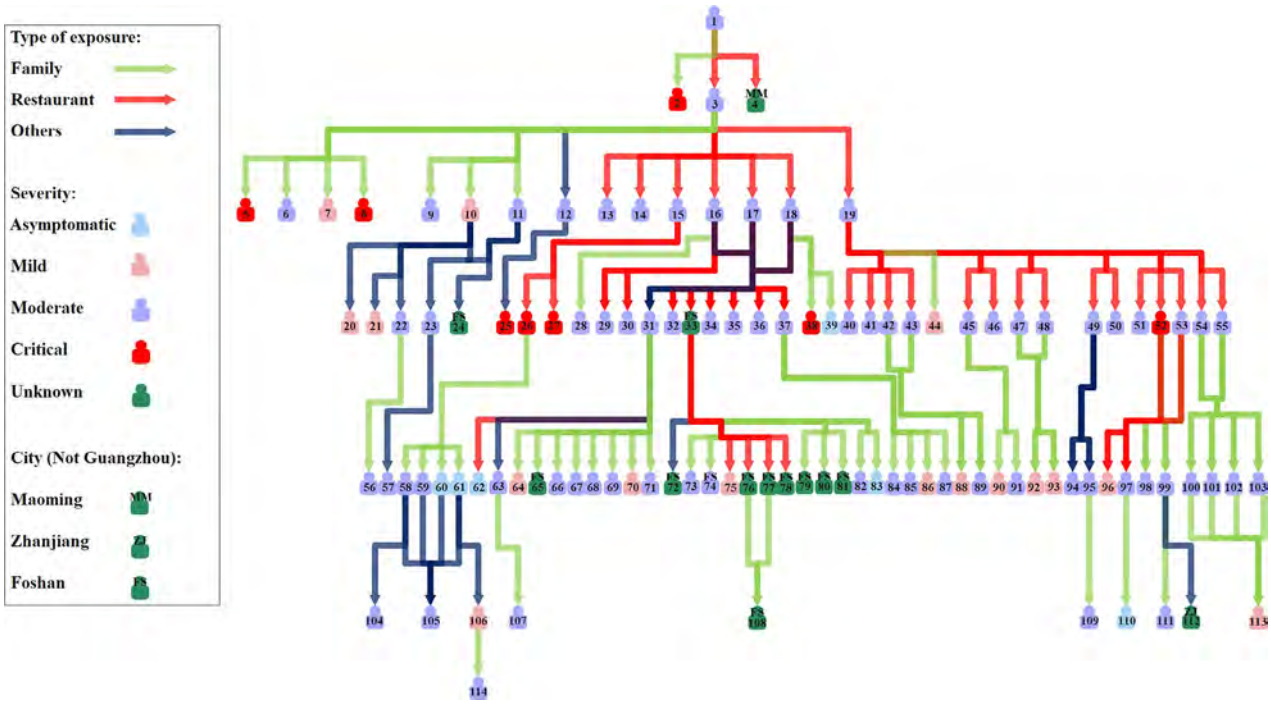


Fig 2. Transmission network of the infections of the SARS-CoV-2 Delta variant. A total of 101 and 13 cases reported in Guangzhou and other cities with information for determining the generation are presented. Cases without a clear epidemiological link with the confirmed cases and the ones whose infector did not have a clear exposure history were not included.

https://doi.org/10.1371/journal.pntd.0010048.g002

one of Case #3's friends (Fig 2). In this outbreak, a total of seven generations were found to be associated with the transmission chain initiated by the first infection of the Delta variant (Fig 2). The number of cases increased gradually from the start of this outbreak and peaked on 1 June with 16 residents showing symptoms or testing positive for SARS-CoV-2 on that day. Thereafter, the number of cases fluctuated and showed a decreasing trend (Fig 1). From 19 June through 24 June 2021, no local case has been reported in Guangzhou.

From 21 May to 24 June 2021, there were 153 local cases reported in Guangzhou (symptomatic cases: 146 [95.4%]; asymptomatic infections: 7 [4.6%]). The median age of the local cases was 48 (range: 1–94) years, and males accounted for 41.2% of these cases (Table 1). More than half of the cases were people who had retired and the unemployed. Preschool children, students, healthcare workers, and others represented 3.3%, 16.3%, 2.6%, and 26.8% of the local cases, respectively. During the study period, 24 (15.7%), 113 (73.9%), 0 (0.0%), and 9 (5.9%) of the patients had mild, moderate, severe, and critical disease severity, respectively (Table 1).

We identified 103 cases with a clear exposure history: 53 (51.5%) were observed within family households, 36 (35.0%) took place in restaurants, and 14 (13.6%) were linked via other exposures (Table 1). Results suggested that the gamma distribution fitted best to the incubation period in terms of AIC (S2 Table). The mean and median incubation periods and were 6.50 (95% confidence interval [CI]: 5.86–7.20) and 6.02 (95% CI: 5.42–6.71) days, respectively. The 95th percentile of the incubation periods was 12.27 (95% CI: 10.68–13.84) days. As for the serial interval, the mean and standard deviation were 4.24 (95% CI: 3.35–5.14) and 3.95 (95% CI: 3.23–4.61) days, respectively (Fig 3) for the entire study period. In addition, we found that the means of serial intervals of different time windows decreased gradually from 5.19 (95% CI:

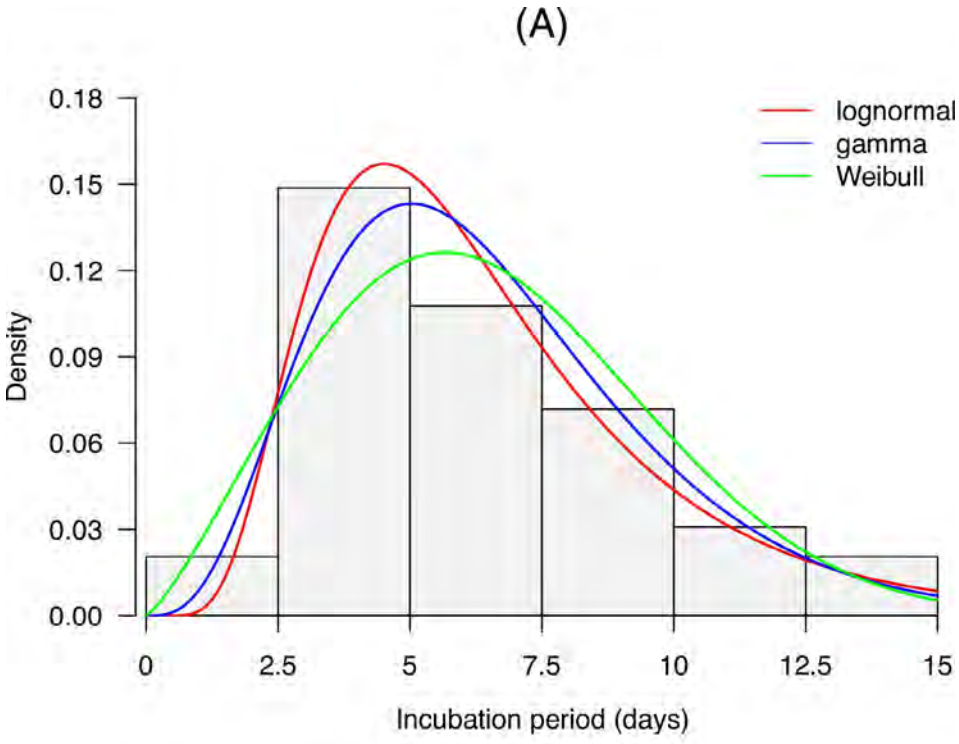
Table 1. The characteristics of the COVID-19 cases in Guangzhou, China, reported from 21 May through 24 June 2021.

Characteristics	Cases (n = 153)
Male sex—no. (%)	63/153 (41.2)
Median age (range)—years	48 (1, 94)
Age group (years)—no. (%)	
≤18	28/153 (18.3)
19–59	72/153 (47.1)
60–70	19/153 (12.4)
≥70	34/153 (22.2)
Occupation—no. (%)	
People who have retired at home and the unemployed	78/153 (51.0)
Preschool children	5/153 (3.3)
Students	25/153 (16.3)
Healthcare workers	4/153 (2.6)
Others	41/153 (26.8)
Type of exposure—no. (%)	
Family	53/103 (51.5)
Exposure to the same restaurant with a confirmed case	36/103 (35.0)
Others	14/103 (13.6)
Type of detection—no. (%)	
Tracing of close contacts	99/153 (64.7)
Mass screening	46/153 (30.1)
Hospital screening	8/153 (5.2)
Clinical severity—no. (%)	
Asymptomatic	7/153 (4.6)
Mild	24/153 (15.7)
Moderate	113/153 (73.9)
Severe	0/153 (0.0)
Critical	9/153 (5.9)

https://doi.org/10.1371/journal.pntd.0010048.t001

4.29–6.11) to 3.78 (95% CI: 2.74–4.81) days (S3 Table). The incubation period was positively associated with age ($P<0.001$, S4 Table), while the associations between age (of infector and infectee) and serial interval were statistically non-significant (S5 and S6 Tables).

In response to the COVID-19 outbreak, the local government formulated a hierarchical prevention and control strategy to suppress community transmission. Generally speaking, Guangzhou was divided into three areas according to the risk level of SARS-CoV-2 transmission. The core areas were the cluster areas in which many COVID-19 cases were reported. The warning zones were the places in which sporadic cases have been found. Other areas were low-risk areas. The level of response to COVID-19 increased with the risk level, with the most rigorous interventions taking place in the areas with the highest level of transmission risk. A series of NPIs and vaccinations were implemented during this outbreak (Fig 1 and S7 Table). Notably, one of the most important measures was case finding through mass tests for COVID-19 among residents in the core areas, warning zones and then the low-risk areas. By 6 June 2021, the entire population of the city had been tested for COVID-19. As of 12 June, over 36 million samples had been collected for SARS-CoV-2 tests. In the core areas and warning zones, multiple rRT-PCR tests have been performed. Vaccination is another important measure for the containment of COVID-19. On 31 May, mass vaccination was stopped and the focus was shifted to case finding through mass tests for COVID-19. However, vaccination was


(A)
lognormal
gamma
Weibull
Density
0.18
0.15
0.12
0.09
0.06
0.03
0.00
0
2.5
5
7.5
10
12.5
15
Incubation period (days)

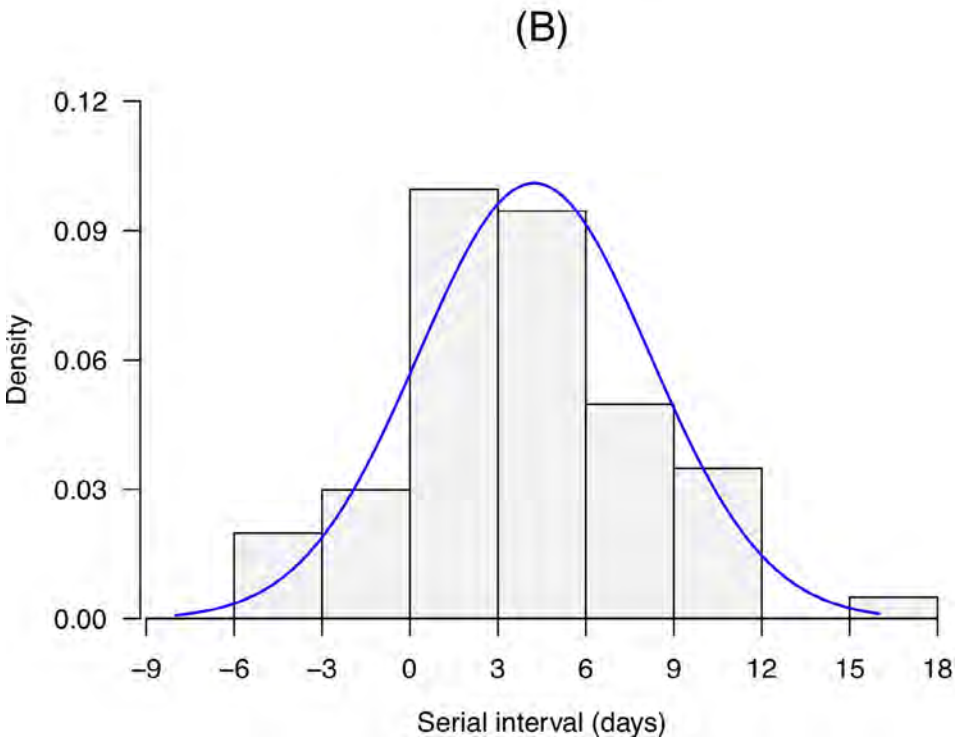

(B)
Density
0.12
0.09
0.06
0.03
0.00
−9
−6
−3
0
3
6
9
12
15
18
Serial interval (days)

Fig 3. Incubation period and serial interval distributions of the SARS-CoV-2 Delta variant in Guangzhou, China. The blue lines represent the estimated distribution densities. Data of 78 cases and 67 transmission pairs were used to estimate the incubation period and serial interval distributions, respectively.

https://doi.org/10.1371/journal.pntd.0010048.g003

restarted on 6 June for individuals who did not live in the core areas and had received one shot 21 days before 6 June. By 24 June, 10.77 million residents had been vaccinated, among whom, 8.72 million had been fully vaccinated. Other interventions included quarantine for high-risk groups, rigorous inspection (e.g. requiring residents to show health codes, measuring body temperature), requiring wearing masks, limiting public gatherings, etc (S7 Table). In this outbreak, 99 cases (64.7%) were in close contact with confirmed cases, while 46 (30.1%) were detected through mass screening (Table 1). With these efforts, R_t decreased rapidly from 6.83 (95% credible interval [CrI]: 3.98–10.44) for the 7-day time window ending on 27 May 2021 to below 1 for the time window ending on 8 June and thereafter (Fig 1).

We found that 21 cases were partially or fully vaccinated before infection (15.3%) among the 137 cases (excluding the 16 cases with indeterminate vaccination status, Table 2). Clinical symptoms were milder in the partially or fully vaccinated cases than the unvaccinated group (odds ratio [*OR*] = 0.26 [95% CI: 0.07–0.94], Table 3). Notably, no critical cases were observed in those who had been partially or fully vaccinated, while 9/116 of the unvaccinated cases were critical cases (Table 2).

Results of sensitivity analysis suggested that the estimates of mean, median and 95th percentile of incubation periods were similar to the ones in the main analysis (S8 Table). The associations of incubation period with occupation and type of exposure were statistically significant in bivariate regression models (S9 Table). Age was positively associated with incubation period in the model with an additional inclusion of occupation and the one with type of exposure (S10 and S11 Tables).

Discussion

In this study, we provided a detailed description of the first community transmission of the SARS-CoV-2 Delta VOC in Guangzhou, China, providing important epidemiological parameters of this outbreak. We found that 4.6% of the cases during the study period were asymptomatic, a figure lower than the 15.6% reported in a previous systematic review [18]. The difference in age structure and definitions of asymptomatic and symptomatic cases may explain the variation in the proportion of asymptomatic infections. We estimated that the mean and median incubation periods were 6.50 and 6.02 days, respectively, which were slightly longer than the pooled estimates of the mean (6.3 days) and median incubation periods (5.4 days) of preexisting strains reported in a systematic review and meta-analysis [19]. The

Table 2. Clinical severity of COVID-19 cases by vaccination status.

Clinical severity	Unvaccinated (n = 116)	Partially or fully vaccinated (n = 21)
Asymptomatic	6 (5.2)	1 (4.8)
Mild	19 (16.4)	5 (23.8)
Moderate	82 (70.7)	15 (71.4)
Severe	0 (0.0)	0 (0.0)
Critical	9 (7.8)	0 (0.0)

Note. Numbers in brackets were proportions. 16 cases with indeterminate vaccination status (infection occurred <21 days after dose 1 or the time interval between infection date and vaccination date was unclear) were excluded.

https://doi.org/10.1371/journal.pntd.0010048.t002

Table 3. Results of an ordinal logistic regression model assessing the association between vaccination status and clinical severity.

Variables	Odds ratio (95% confidence interval)	*t*	*P*
Age	1.11 (1.08–1.15)	5.940	<0.001
Vaccination status			
Unvaccinated	Reference		
Partially or fully vaccinated	0.26 (0.07–0.94)	-2.025	0.043

Note. Sample size was 137.

https://doi.org/10.1371/journal.pntd.0010048.t003

difference may be due to not only the biological discrepancy in the circulating strains, but also the definitions of symptom onset date and possible infection date, and the approach of estimation [19,20,21,22]. Consistent with a prior study in Singapore [21], we found that the incubation period was positively associated with age. The longer incubation period observed in the old cases probably resulted from a slower immune response in the elderly [21,23]. The higher proportion of old cases (22.2% of the local cases were aged 70 years and older) in this outbreak may in part contribute to a longer incubation period than that for the transmission in 2020 in 30 provinces of China [24]. Older age of the subjects in the present study may also explain why our estimate of the mean of incubation period was larger than 5.8 days which was reported in a study of the Delta variant [25]. We found that the maximum incubation period was 15 days, which indicated that longer quarantine periods (>14 days) would be required for extreme cases [26].

Seven generations were found to be associated with the transmission chain initiated by the first infection of the Delta variant in approximately 20 days, which indicated that this variant may be transmitted rapidly. A previous study in the United Kingdom reported that the household transmission rate associated with the Delta variant was higher than that of the Alpha variant, which was found to have a 43–90% higher reproductive number than the preexisting strains [27,28]. In England, the first confirmed case of the Delta variant was detected in late March 2021, and this variant accounted for more than 90% of all new cases at the end of May 2021 [28,29], which also suggested its potential for high transmissibility. Our study estimated that the mean and standard deviation of serial intervals were 4.24 and 3.95 days, respectively for the entire study period. A substantial fraction of secondary transmission was likely to occur prior to illness onset given the shorter serial interval compared with the incubation period [30]. Our estimate of the mean serial interval was larger than that for the strains circulating in early 2020 in China (3.66 days for the locally infected) [14] and the Delta variant circulating in Daejeon, South Korea (3.26 days) [31]. In addition, we estimated that the means of serial intervals of different time windows decreased from 5.19 to 3.78 days. Shorten estimates of means of serial intervals over time were also reported in previous studies [17,25]. The estimate of R_t is influenced by the mean and standard deviation of serial interval. A larger mean of serial interval may lead to a higher R_t, while a larger standard deviation may result in a R_t which is closer to 1 [17]. Therefore, estimating R_t for the Delta VOC using the estimate of preexisting strains may introduce bias.

In this study, we estimated the R_t based on the time-varying distributions of serial intervals and found that R_t declined from 6.83 for the time window ending on 27 May 2021 to below 1 for the time window ending on 8 June and thereafter, which suggested that the interventions in Guangzhou were timely and effective. It is worth noting that the estimated R_t should be interpreted in the context of reduced transmission with great efforts, including social distancing interventions and mass vaccination programs in Guangzhou.

In this outbreak, 94.8% of COVID-19 cases were detected among close contacts of confirmed cases and through mass screening of residents. This finding suggests that case finding through mass tests for COVID-19 and case isolation are of great importance for the control of COVID-19 when the implementation is feasible. It is recommended to implement mass screening to detect the COVID-19 cases when some cases of unknown origin occur and it seems that the pathogen spreads.

Vaccination is an important intervention for the prevention and control of infectious diseases. Randomized-controlled trials and observational studies have revealed vaccine efficacy/effectiveness ranging from 50–95% against symptomatic COVID-19 caused by preexisting strains, including the Alpha variant [10,32,33]. A recent study in the United States indicated that the adjusted effectiveness of the authorised mRNA vaccines in preventing SARS-CoV-2 infection was 91% and 81% with full vaccination and partial vaccination, respectively, when administered in real-world conditions [34]. In Chile, the effectiveness of CoronaVac was 65.9%, 87.5%, and 90.3% for the prevention of infection, hospitalization, and ICU admission for the individuals with fully immunized [35]. In Guangzhou, the vaccination coverage of the whole population (67%) was approximately 2.4 times higher than the coverage of COVID-19 cases (15.3%). In this study, we found that the partially or fully vaccinated cases generally had milder symptoms than those in the unvaccinated group after controlling for age. In addition, Li et al. conducted a test-negative case-control study to assess the effectiveness of inactivated vaccines among residents aged 18–59 in Guangzhou using the close contacts of confirmed cases as controls [36]. Results suggested that the overall vaccine effectiveness for two-dose vaccination was 59.0% against COVID-19 and 70.2% against moderate COVID-19. These data further implied that the authorised inactivated vaccines are probably capable of protecting people from the Delta VOC, and vaccination can reduce the probability of the occurrence of severe disease. In Guangzhou, the target population of vaccination was mainly residents aged 18–59 years without contraindications during the study period. Currently, the vaccination is free for residents aged 12 years of age and older in China, as more evidence has proved that the authorised inactivated COVID-19 vaccines are safe and effective [37–40]. Mass screening and vaccination are labour-intensive, especially when the two measures are implemented at the same time. In China, community health centers and hospitals organize the mass screening and vaccination, with great support from volunteers.

We found that 37 vaccinated individuals were infected in this outbreak. Vaccine breakthrough infections were also reported in other locations [41,42,43]. Nevertheless, the vaccine breakthrough infections only occurred in a small percentage of vaccinated individuals, meanwhile, these cases merely represented a small fraction of COVID-19 cases [41]. COVID-19 vaccination is still an effective measure to prevent infection, severe illness, and death [42]. Given that the infections can occur in vaccinated individuals, personal protection measures, such as wearing masks in indoor public settings where the transmission risk of COVID-19 is high, are still needed [42].

We found that 51.5% of the transmission pairs had a family bound. Consistently, transmission within family households was the most frequent in the first wave of COVID-19 in Guangzhou and Hong Kong [44,45]. SARS-CoV-2 transmission in restaurants has been reported previously [46]. Improving ventilation and increasing the distance between tables may reduce the infection risk [46]. Eating at restaurants was restricted in this outbreak, which has in part mitigated the transmission of COVID-19.

Our study had some limitations. First, our analysis mainly focused on the characteristics of the cases of SARS-CoV-2 infection reported in Guangzhou, since some important information (e.g. symptom onset date, clinical severity, and vaccination status) of the cases reported in other cities was not available. Second, the infection and symptom onset dates were reported by

the patients and the infection dates were not clear for some COVID-19 cases. Also, some transmission pairs were not determined. Potential bias may influence the estimates of the incubation period, serial interval, and R_t. Third, we did not account for pre-symptomatic transmission when estimating R_t. This will be addressed in future studies. Next, we did not evaluate a specific intervention in this study but the combination of various control measures, since these interventions were implemented simultaneously, and it was difficult to distinguish their effects. In addition, it would be more informative if averted number of COVID-19 cases attributable to the interventions can be provided. Further studies will quantify the effects using mathematical and statistical models. Last, possibly insufficient sample size can affect the statistical power and the conclusion. For instance, the sample size for the inference of the effect of vaccination status on clinical severity may be not sufficient. More solid evidence will be available with real-world data from a large sample size.

In conclusion, the hierarchical prevention and control strategy against COVID-19 in Guangzhou was timely and effective. Case finding through mass tests for COVID-19 and case isolation are important for the containment of SARS-CoV-2 transmission if the implementation is feasible. Receiving the authorised inactivated vaccines may reduce the probability of developing severe disease after infection. It is recommended that eligible individuals be vaccinated to better protect themselves against COVID-19. Our findings have important implications for the containment of COVID-19.

Supporting information

S1 File. Real-time reverse transcription-polymerase chain reaction.
(DOCX)

S1 Fig. Data on incubation period and serial interval used in the main analysis.
(TIF)

S1 Table. Definitions of cases with different clinical severity.
(XLSX)

S2 Table. Values of Akaike Information Criteria (AIC) for three distributions fitted to incubation periods.
(XLSX)

S3 Table. Estimates of means and standard deviations of serial intervals for different time windows.
(XLSX)

S4 Table. Results of the model which assessed the association between age and incubation period in the main analysis.
(XLSX)

S5 Table. Results of the model which examined the association between age of infector and serial interval.
(XLSX)

S6 Table. Results of the model which evaluated the association between age of infectee and serial interval.
(XLSX)

S7 Table. Interventions for the areas of different transmission risk of SARS-CoV-2.
(XLSX)

S8 Table. Estimates of the means, medians and 95th percentiles of incubation periods in the sensitivity analysis.
(XLSX)

S9 Table. Results of bivariate regression models for incubation period.
(XLSX)

S10 Table. Results of the model which assessed the association between age and incubation period with an adjustment of occupation.
(XLSX)

S11 Table. Results of the model which examined the association between age and incubation period with an adjustment of type of exposure.
(XLSX)

Acknowledgments

We thank staff members of Guangzhou Center for Disease Control and Prevention for administrative work and data collection.

Author Contributions

Conceptualization: Chun-Quan Ou, Lei Luo.

Data curation: Zhi-Gang Han, Peng-Zhe Qin, Wen-Hui Liu, Zong-Qiu Chen, Ke Li, Chao-Jun Xie, Yu Ma, Hui Wang, Yong Huang, Shu-Jun Fan.

Formal analysis: Li Li, Zhou Yang, Ze-Lin Yan.

Funding acquisition: Li Li, Chun-Quan Ou, Lei Luo.

Investigation: Chun-Quan Ou, Lei Luo.

Methodology: Li Li, Chun-Quan Ou, Lei Luo.

Project administration: Wen-Hui Liu.

Supervision: Chun-Quan Ou, Lei Luo.

Writing – original draft: Li Li, Zhi-Gang Han, Peng-Zhe Qin, Wen-Hui Liu.

Writing – review & editing: Li Li, Zhi-Gang Han, Peng-Zhe Qin, Wen-Hui Liu, Zhou Yang, Zong-Qiu Chen, Ke Li, Chao-Jun Xie, Yu Ma, Hui Wang, Yong Huang, Shu-Jun Fan, Ze-Lin Yan, Chun-Quan Ou, Lei Luo.

References

1. World Health Organization. Weekly epidemiological update on COVID-19–12 July 2021. [Cited 12 July 2021]. Available from: https://www.who.int/publications/m/item/weekly-operational-update-on-covid-19---12-july-2021.
2. Hamamoto Y. The Covid-19 world-Are we there yet? J Diabetes Investig. 2021; 12(7):1125–7. https://doi.org/10.1111/jdi.13605 PMID: 34056843
3. Ranjan R, Sharma A, Verma MK. Characterization of the Second Wave of COVID-19 in India. medRxiv. 2021:2021.04.17.21255665.
4. World Health Organization. Tracking SARS-CoV-2 variants. [Cited 26 June 2021]. Available from: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/.
5. Public Health England. SARS-CoV-2 variants of concern and variants under investigation in England—Technical briefing 17. [Cited 26 June 2021]. Available from: https://assets.publishing.service.gov.uk/

government/uploads/system/uploads/attachment_data/file/996740/Variants_of_Concern_VOC_Technical_Briefing_17.pdf.

6. Liu K, Ai S, Song S, Zhu G, Tian F, Li H, et al. Population Movement, City Closure in Wuhan, and Geographical Expansion of the COVID-19 Infection in China in January 2020. Clin Infect Dis. 2020; 71 (16):2045–51. https://doi.org/10.1093/cid/ciaa422 PMID: 32302377
7. Zhang ZB, Li L, Qin PZ, Li K, Huang Y, Luo L, et al. Countries of origin of imported COVID-19 cases into China and measures to prevent onward transmission. J Travel Med. 2020; 27(8):taaa139. https://doi.org/10.1093/jtm/taaa139 PMID: 32841347
8. Guangzhou Municipal Health Commission. COVID-19 situation update in Guangzhou on 21 May 2021. [Cited 26 June 2021]. Available from: http://wjw.gz.gov.cn/ztzl/xxfyyqfk/yqtb/content/post_7295197.html.
9. US Centers for Disease Control and Prevention. Delta Variant: What We Know About the Science. [Cited 9 September 2021]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/variants/delta-variant.html.
10. Patel MK, Bergeri I, Bresee JS, Cowling BJ, Crowcroft NS, Fahmy K, et al. Evaluation of post-introduction COVID-19 vaccine effectiveness: Summary of interim guidance of the World Health Organization. Vaccine. 2021; 39(30):4013–24. https://doi.org/10.1016/j.vaccine.2021.05.099 PMID: 34119350
11. The People's Govenment of Guangzhou Municipality. Suspending social vaccination against COVID-19 and concentrating on case findings. [Cited 26 June 2021]. Available from: http://www.gz.gov.cn/zt/qlyfdyyqfkyz/gzzxd/content/post_7308978.html.
12. National Health Commission of the People's Republic of China. Diagnosis and treatment protocols of pneumonia caused by a novel coronavirus (trial version 8). [Cited 26 June 2021]. Available from: http://www.gov.cn/zhengce/zhengceku/2020-08/19/5535757/files/da89edf7cc9244fbb34ecf6c61df40bf.pdf.
13. Ryu S, Ali ST, Noh E, Kim D, Lau EHY, Cowling BJ. Transmission dynamics and control of two epidemic waves of SARS-CoV-2 in South Korea. BMC Infect Dis. 2021; 21(1):485. https://doi.org/10.1186/s12879-021-06204-6 PMID: 34039296
14. Du Z, Xu X, Wu Y, Wang L, Cowling BJ, Meyers LA. Serial Interval of COVID-19 among Publicly Reported Confirmed Cases. Emerg Infect Dis. 2020; 26(6):1341–3. https://doi.org/10.3201/eid2606.200357 PMID: 32191173
15. Cori A, Ferguson NM, Fraser C, Cauchemez S. A new framework and software to estimate time-varying reproduction numbers during epidemics. Am J Epidemiol. 2013; 178(9):1505–12. https://doi.org/10.1093/aje/kwt133 PMID: 24043437
16. Wallinga J, Lipsitch M. How generation intervals shape the relationship between growth rates and reproductive numbers. Proc Biol Sci. 2007; 274(1609):599–604. https://doi.org/10.1098/rspb.2006.3754 PMID: 17476782
17. Ali ST, Wang L, Lau EHY, Xu XK, Du Z, Wu Y, et al. Serial interval of SARS-CoV-2 was shortened over time by nonpharmaceutical interventions. Science. 2020; 369(6507):1106–9. https://doi.org/10.1126/science.abc9004 PMID: 32694200
18. He J, Guo Y, Mao R, Zhang J. Proportion of asymptomatic coronavirus disease 2019: A systematic review and meta-analysis. J Med Virol. 2021; 93(2):820–30. https://doi.org/10.1002/jmv.26326 PMID: 32691881
19. Xin H, Wong JY, Murphy C, Yeung A, Ali ST, Wu P, et al. The incubation period distribution of coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis. Clin Infect Dis. 2021; https://doi.org/10.1093/cid/ciab501 PMID: 34117868
20. Wang M, Li M, Ren R, Li L, Chen EQ, Li W, et al. International Expansion of a Novel SARS-CoV-2 Mutant. J Virol. 2020; 94(12):e00567–20. https://doi.org/10.1128/JVI.00567-20 PMID: 32269121
21. Tan WYT, Wong LY, Leo YS, Toh M. Does incubation period of COVID-19 vary with age? A study of epidemiologically linked cases in Singapore. Epidemiol Infect. 2020; 148:e197. https://doi.org/10.1017/S0950268820001995 PMID: 32873357
22. Park SW, Sun K, Champredon D, Li M, Bolker BM, Earn DJD, et al. Forward-looking serial intervals correctly link epidemic growth to reproduction numbers. PNSA. 2021; 118(2):e2011548118. https://doi.org/10.1073/pnas.2011548118 PMID: 33361331
23. Cowling BJ, Muller MP, Wong IO, Ho LM, Louie M, McGeer A, et al. Alternative methods of estimating an incubation distribution: examples from severe acute respiratory syndrome. Epidemiology. 2007; 18 (2):253–9. https://doi.org/10.1097/01.ede.0000254660.07942.fb PMID: 17235210
24. Zhang J, Litvinova M, Wang W, Wang Y, Deng X, Chen X, et al. Evolving epidemiology and transmission dynamics of coronavirus disease 2019 outside Hubei province, China: a descriptive and modelling study. Lancet Infect Dis. 2020; 20(7):793–802. https://doi.org/10.1016/S1473-3099(20)30230-9 PMID: 32247326

25. Kang M, Xin H, Yuan J, Ali ST, Liang Z, Zhang J, et al. Transmission dynamics and epidemiological characteristics of Delta variant infections in China. medRxiv. 2021:2021.08.12.21261991.

26. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med. 2020; 172(9):577–82. https://doi.org/10.7326/M20-0504 PMID: 32150748

27. Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Munday JD, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science. 2021; 372(6538):eabg3055. https://doi.org/10.1126/science.abg3055 PMID: 33658326

28. Allen H, Vusirikala A, Flannagan J, Twohig KA, Zaidi A, COG-UK Consortium, et al. Increased household transmission of COVID-19 cases associated with SARS-CoV-2 Variant of Concern B.1.617.2: a national case-control study. [Cited 26 June 2021]. Available from: https://khub.net/documents/135939561/405676950/Increased+Household+Transmission+of+COVID-19+Cases+-+national+case+study.pdf/7f7764fb-ecb0-da31-77b3-b1a8ef7be9aa.

29. Public Health England. SARS-CoV-2 variants of concern and variants under investigation in England: Technical briefing 14. [Cited 26 June 2021]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/991343/Variants_of_Concern_VOC_Technical_Briefing_14.pdf.

30. Nishiura H, Linton NM, Akhmetzhanov AR. Serial interval of novel coronavirus (COVID-19) infections. Int J Infect Dis. 2020; 93:284–6. https://doi.org/10.1016/j.ijid.2020.02.060 PMID: 32145466

31. Hwang H, Lim J-S, Song S-A, Achangwa C, Sim W, Ryu S, et al. Transmission dynamics of the delta variant of SARS-CoV-2 infections in Daejeon, South Korea. [Cited November 11, 2021]. Available from: https://assets.researchsquare.com/files/rs-934350/v1/26a74aee-d71b-4c22-b04f-df95a8def2c8.pdf?c=1633023281.

32. Lopez Bernal J, Andrews N, Gower C, Robertson C, Stowe J, Tessier E, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. BMJ. 2021; 373:n1088. https://doi.org/10.1136/bmj.n1088 PMID: 33985964

33. Pritchard E, Matthews PC, Stoesser N, Eyre DW, Gethings O, Vihta KD, et al. Impact of vaccination on new SARS-CoV-2 infections in the United Kingdom. Nat Med. 2021; 27(8):1370–8. https://doi.org/10.1038/s41591-021-01410-w PMID: 34108716

34. Thompson MG, Burgess JL, Naleway AL, Tyner H, Yoon SK, Meece J, et al. Prevention and Attenuation of Covid-19 with the BNT162b2 and mRNA-1273 Vaccines. NEJM. 2021; 385(4):320–9. https://doi.org/10.1056/NEJMoa2107058 PMID: 34192428

35. Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. NEJM. 2021; 385(10):875–84. https://doi.org/10.1056/NEJMoa2107715 PMID: 34233097

36. Li XN, Huang Y, Wang W, Jing QL, Zhang CH, Qin PZ, et al. Effectiveness of inactivated SARS-CoV-2 vaccines against the Delta variant infection in Guangzhou: a test-negative case-control real-world study. Emerg Microbes Infect. 2021; 10(1):1751–9. https://doi.org/10.1080/22221751.2021.1969291 PMID: 34396940

37. Mallapaty S. WHO approval of Chinese CoronaVac COVID vaccine will be crucial to curbing pandemic. Nature. 2021; 594(7862):161–2. https://doi.org/10.1038/d41586-021-01497-8 PMID: 34089030

38. Al Kaabi N, Zhang Y, Xia S, Yang Y, Al Qahtani MM, Abdulrazzaq N, et al. Effect of 2 Inactivated SARS-CoV-2 Vaccines on Symptomatic COVID-19 Infection in Adults: A Randomized Clinical Trial. JAMA. 2021; 326(1):35–45. https://doi.org/10.1001/jama.2021.8565 PMID: 34037666

39. Wu Z, Hu Y, Xu M, Chen Z, Yang W, Jiang Z, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021; 21(6):803–12. https://doi.org/10.1016/S1473-3099(20)30987-7 PMID: 33548194

40. Xia S, Zhang Y, Wang Y, Wang H, Yang Y, Gao GF, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. Lancet Infect Dis. 2021; 21(1):39–51. https://doi.org/10.1016/S1473-3099(20)30831-8 PMID: 33069281

41. US CDC COVID-19 Vaccine Breakthrough Case Investogations Team. COVID-19 Vaccine Breakthrough Infections Reported to CDC—United States, January 1-April 30, 2021. MMWR. 2021; 70(21):792–3. https://doi.org/10.15585/mmwr.mm7021e3 PMID: 34043615

42. Brown CM, Vostok J, Johnson H, Burns M, Gharpure R, Sami S, et al. Outbreak of SARS-CoV-2 Infections, Including COVID-19 Vaccine Breakthrough Infections, Associated with Large Public Gatherings—Barnstable County, Massachusetts, July 2021. MMWR. 2021; 70(31):1059–62. https://doi.org/10.15585/mmwr.mm7031e2 PMID: 34351882

43. Brosh-Nissimov T, Orenbuch-Harroch E, Chowers M, Elbaz M, Nesher L, Stein M, et al. BNT162b2 vaccine breakthrough: clinical characteristics of 152 fully vaccinated hospitalized COVID-19 patients in Israel. Clin Microbiol Infect. 2021; https://doi.org/10.1016/j.cmi.2021.06.036 PMID: 34245907

44. Jing QL, Liu MJ, Zhang ZB, Fang LQ, Yuan J, Zhang AR, et al. Household secondary attack rate of COVID-19 and associated determinants in Guangzhou, China: a retrospective cohort study. Lancet Infect Dis. 2020; 20(10):1141–50. https://doi.org/10.1016/S1473-3099(20)30471-0 PMID: 32562601

45. Adam DC, Wu P, Wong JY, Lau EHY, Tsang TK, Cauchemez S, et al. Clustering and superspreading potential of SARS-CoV-2 infections in Hong Kong. Nat Med. 2020; 26(11):1714–9. https://doi.org/10.1038/s41591-020-1092-0 PMID: 32943787

46. Lu J, Gu J, Li K, Xu C, Su W, Lai Z, et al. COVID-19 Outbreak Associated with Air Conditioning in Restaurant, Guangzhou, China, 2020. Emerg Infect Dis. 2020; 26(7):1628–31. https://doi.org/10.3201/eid2607.200764 PMID: 32240078

6.9. CoronaVac em adolescentes com doenças reumáticas causa três vezes menos efeitos adversos do que vacinas de RNA mensageiro

Um grupo de pesquisadores da Faculdade de Medicina da Universidade de Istambul, na Turquia, concluiu que em jovens que recebem a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, o índice de efeitos adversos após a imunização é três vezes menor do que em quem toma vacinas feitas com a tecnologia de RNA mensageiro. O resultado foi descrito em estudo publicado no International Journal of Rheumatic Diseases, e baseado no acompanhamento, ao longo de um ano, de 246 adolescentes com idade média de 15 anos.

Dos 145 participantes da pesquisa que haviam tomado a vacina de RNA mensageiro, 107 (74%) experimentaram eventos adversos relacionados à imunização. Dos 32 que tomaram CoronaVac, apenas sete (22%) relataram efeitos adversos. Os sintomas mais comuns foram fadiga, cefaleia, mialgia, artralgia e febre.

Três indivíduos relataram eventos adversos graves, uma vez que necessitaram de hospitalização e tratamento adicional. Uma garota de 20 anos desenvolveu hipertensão após a segunda dose, uma garota de 12 anos apresentou erupção cutânea grave após a primeira dose, e um adolescente de 13 desenvolveu pré-síncope por hipotensão após a primeira dose. Nenhum deles havia tomado CoronaVac.

Esses resultados comprovam, novamente, que a vacina do Butantan e da Sinovac é a que tem o melhor perfil de segurança dentre os imunizantes atualmente em uso contra a Covid-19, seja em adultos, idosos, crianças ou adolescentes.

No grupo investigado havia 126 pacientes com doenças autoinflamatórias, 54 pacientes com artrite idiopática juvenil, 30 pacientes com doença do tecido conjuntivo, nove com vasculite e quatro com febre reumática aguda. O grupo controle foi composto por 23 adolescen-

tes saudáveis. Dos voluntários, 214 pacientes receberam a vacina de RNA mensageiro, 28 tomaram a CoronaVac e quatro tomaram as duas. Antes da imunização, 44 indivíduos haviam contraído Covid-19 e se recuperado, sendo que quatro deles apresentaram infecção assintomática e o restante só sintomas leves. A grande maioria tomava regularmente medicação antes da imunização e continuou após receber a vacina.

De acordo com os pesquisadores, "nosso estudo indica um perfil de segurança aceitável das vacinas contra Covid-19 disponíveis em nosso país [Turquia] e incentiva as crianças com doenças reumáticas a serem vacinadas".

Nos primeiros dias da pandemia, as crianças eram consideradas como tendo um curso assintomático ou leve de Covid-19, em contraste com os adultos. No entanto, um número crescente de casos pediátricos com síndrome inflamatória multissistêmica em crianças, causada pelo SARS-CoV-2, têm sido descritos com consequências devastadoras, como internação em unidade de terapia intensiva ou até óbito. Portanto, estratégias de vacinação precisam ser bem estabelecidas para crianças, assim como para adultos.

Publicado em: 3/1/2022

Received: 5 November 2021 | Revised: 21 December 2021 | Accepted: 22 December 2021

DOI: 10.1111/1756-185X.14279

International Journal of Rheumatic Diseases

WILEY

ORIGINAL ARTICLE

Early experience of COVID-19 vaccine-related adverse events among adolescents and young adults with rheumatic diseases: A single-center study

Fatih Haslak | Aybuke Gunalp | Memnune Nur Cebi | Mehmet Yildiz | Amra Adrovic | Sezgin Sahin | Kenan Barut | Ozgur Kasapcopur

Department of Pediatric Rheumatology, Istanbul University-Cerrahpasa Cerrahpasa Medical School, Istanbul, Turkey

Correspondence
Ozgur Kasapcopur, Department of Pediatric Rheumatology, Istanbul University-Cerrahpasa, Cerrahpasa Medical School, Istanbul, Turkey.
Email: ozgurkasapcopur@hotmail.com

Abstract

Objective: Considering the concerns regarding the coronavirus disease-2019 (COVID-19) vaccine safety among pediatric patients with inflammatory rheumatic diseases (IRD) due to a lack of data, an urgent need for studies evaluating safety profiles of vaccines emerged.

Methods: Among participants vaccinated by CoronaVac inactive SARS-CoV-2 or BNT162b2 messenger RNA (mRNA) COVID-19 (Pfizer-BioNTech) vaccine, healthy children under 18 and patients under 21 with an at least 1-year follow-up period in our department for a childhood-onset rheumatic disease were included into this cross-sectional study.

Results: Overall, 246 subjects (141 [57.3%] females) (biologic group: 43, non-biologic group: 180, healthy control group: 23) were eligible for the study. The median age was 15.34 (12.02-20.92) years. The most common adverse events were fatigue (n = 68, 27.6%), headache (n = 44, 17.9%), myalgia (n = 38, 15.4%), arthralgia (n = 38, 15.4%), and fever (n = 35, 14.2%). Only 3 subjects (2 patients with familial Mediterranean fever, and one healthy child) were considered to experienced serious adverse events, since they required hospitalization. Local reactions were seen in 20 (8.13%), and 27 patients (12.1%) had disease flares within 1 month after the vaccines. Although it was significantly higher in those who received the BNT162b2 mRNA vaccine ($P < .001$), there was no significant relationship between adverse event frequency and age, gender, the existing diseases, ongoing treatment regimens and pre-vaccination COVID-19 histories.

Conclusion: Although immunogenicity studies for efficacy of the vaccines and long-term follow-up studies for adverse events monitoring are required, our study indicates an acceptable safety profile of COVID-19 vaccines and encourages children with IRD to be vaccinated.

KEYWORDS
COVID-19, pediatrics, rheumatology, SARS-CoV-2, vaccines

1 | INTRODUCTION

For almost 2 years, our planet has been suffering from coronavirus disease-2019 (COVID-19) caused by a novel coronavirus named severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2). Although scientists worldwide are mainly focused on the pandemic, there is still no available therapeutic option that may provide sufficient cure, and COVID-19 remains a significant global health concern. Thus, preventive strategies such as face masks, social distancing, personal hygiene, and vaccination come into prominence. Recently, several studies have shown newly developed vaccines to be effective and safe tools for the fight against COVID-19.[1,2]

In the early days of the pandemic, children were considered to have an asymptomatic or a mild COVID-19 disease course in contrast to adults.[3] However, a growing number of pediatric cases with multi-system inflammatory syndrome in children (MIS-C) caused by SARS-CoV-2 have been described with devastating consequences such as intensive care unit admission or even death.[4,5] Therefore, vaccination strategies are needed to be well-established for children, as well as for adults.

There is a vulnerable group such as immunocompromised patients among the pediatric population that merits to be prioritized for the vaccination. Patients with inflammatory rheumatic diseases (IRD) are considered to be in this group, due to their immune-disturbed conditions caused by their medications and chronic inflammatory states. However, it is still debated whether IRD increases the risk of severe COVID-19 due to conflicting findings of current studies.[6-11]

Although patients with IRD and those under immunosuppressive treatment were mainly excluded from the clinical trials of recent vaccines, they were widely vaccinated.[12] Since they may be at increased risk of worse outcomes from vaccine-preventable diseases, and due to limited source of vaccines in most of the developing countries, they were considered to be a prioritized group by authorities.[13,14] Yet there is no sufficient safety data, particularly for the vaccination of children with IRD.

There are 2 different kinds of COVID-19 vaccines, CoronaVac inactive SARS-CoV-2 and BNT162b2 messenger RNA (mRNA) COVID-19 (Pfizer-BioNTech), which are currently available in our country. Considering the concerns regarding COVID-19 vaccine safety among pediatric patients with IRD due to a lack of data, an urgent need for studies evaluating safety profiles of vaccines emerged. We designed this cross-sectional study to examine the vaccine-related adverse events among this group of patients.

2 | MATERIALS AND METHODS

2.1 | Patients and data collection

In our country, in January 2021, healthcare professionals, and in February 2021, patients with chronic health conditions, those older than 18, were started to be vaccinated by 2 doses of CoronaVac inactive SARS-CoV-2 with a 1-month interval. Afterward, the third to choose their vaccine type, as CoronaVac inactive SARS-CoV-2 or BNT162b2 mRNA COVID-19 (Pfizer-BioNTech). Finally, the fourth dose was approved for both groups in August 2021. Again, individuals were free to prefer their vaccine type.

In mid-August 2021, CoronaVac inactive SARS-CoV-2 and BNT162b2 mRNA COVID-19 vaccines started being administered to children older than 12 with chronic medical conditions and healthy children older than 15 in our country. Then, at the beginning of September 2021, vaccine administration against the novel coronavirus was launched for all children under 12, regardless of their underlying disease.

We conducted a web-based survey in mid-September 2021. Questionnaires regarding the data of the rheumatic diseases, COVID-19 vaccination status, disease flares within 1 month after the vaccines, and experienced adverse events (due to vaccines) of the participants were prepared in Google Forms and circulated through several social media platforms.

Healthy children under 18 and patients under 21 with an at least 1-year follow-up period in our department for a childhood-onset rheumatic disease were included in the study. While data of the rheumatic patients were verified by their medical records, data of COVID-19 vaccination status and experienced adverse events of the participants were verified by phone calls and national registries. Subjects whose data could not be verified by phone calls, registries or medical records were excluded from the study due to a lack of data.

Redness, warmth, regional pain, and tenderness at the injection site due to COVID-19 vaccines were considered as local reactions. While permanent disabilities, hospitalization or an extended hospital stay (if vaccinated while in the hospital), life-threatening illness, birth defects (congenital anomalies), and death were considered severe adverse events, the rest of the adverse events were considered non-severe adverse events, based on the recommendations of Vaccine Adverse Event Reporting System (VAERS) which is co-managed by the Centers for Disease Control and Prevention and the US Food and Drug Administration.[15]

Subjects were categorized into 3 different groups. Children with no underlying disease were considered the healthy control group. While rheumatic patients who were receiving at least one of the biologic agents such as etanercept, infliximab, adalimumab, anakinra, canakinumab, tocilizumab, and rituximab during their vaccination periods were considered the biologic group, the rest of the rheumatic patients were considered the non-biologic group.

The institutional ethics committee of our center approved the study protocol (03/09/21-29430533-903.99-175245). The recommendations of the Declaration of Helsinki for biomedical research involving human subjects were followed. At least one of the family members of all the participants provided informed consent.

2.2 | Statistical analysis

The statistical analysis was performed using SPSS for Windows, version 21.0 (SPSS Inc). Categorical variables were expressed as

(minimum-maximum), based on their distribution which was measured by using the Kolmogorov-Smirnov test. Categorical variables were compared by using Chi-square test or Fisher's exact test, when available. Ages of the patients were compared using the Mann-Whitney *U* or Kruskal-Wallis test, when appropriate. Statistical significance was defined as $P < .05$. Prism software (Prism 8, GraphPad Software) was used to analyze and graph data.

3 | RESULTS

3.1 | Study population

Following the link of our web-based survey that was shared on our clinic's online social media platforms, 466 participants fulfilled the questions. Those who stated that they were not vaccinated (n = 181) were not included in the study. Among those who stated they were vaccinated, those who could not be reached by phone (n = 19), whose follow-up period was <1 year (n = 8) and whose data could not be verified via the national registries, medical records of our department or phone calls (n = 12) were excluded.

Finally, 246 subjects (141 females) were eligible for the study. The median age was 15.34 (12.02-20.92) years. Twenty-three participants whose parents stated in the survey that they did not have any chronic diseases, and whose medical records were checked and confirmed by phone calls that they did not have any underlying disease or long-term medication were considered the healthy control (HC) group.

In the study group there were 126 patients with autoinflammatory diseases (AID) (familial Mediterranean fever [FMF], 123; cryopyrin-associated periodic syndrome [CAPS], 2; Blau syndrome [BS]), 54 patients with juvenile idiopathic arthritis (JIA) (oligoarticular JIA [oJIA], 43; juvenile spondylarthritis [jSPA], 8; polyarticular JIA [pJIA]), 30 patients with connective tissue disease (CTD) (systemic lupus erythematosus [SLE], 16; dermatomyositis [DM], 10; scleroderma, 3; Sjögren's syndrome, 1), 9 patients with vasculitis (Behçet's disease [BD], 2; deficiency of adenosine deaminase 2 [DADA2], 2; Takayasu arteritis [TA], 2; granulomatous polyangiitis [GPA], 1; Henoch-Schönlein purpura [HSP], 2; Kawasaki disease [KD]) and 4 patients with acute rheumatic fever (ARF) (Table 1).

During their vaccination periods, 128 patients were receiving colchicine (FMF, 123; CAPS, 2; BD, 2; DADA2, 1); 49 conventional disease-modifying antirheumatic drugs (cDMARDs) (methotrexate [MTX], 22 [JIA, 12; DM, 7; scleroderma, 2; SLE, 1]; hydroxychloroquine [HCQ], 21 [SLE, 16; DM, 3; Sjögren, 1; scleroderma, 1]; leflunomide, 10 [JIA; 9; SLE, 1]; mycophenolate mofetil [MMF]; 6 [SLE, 3; scleroderma, 2; DM, 1]; cyclosporine; 3 [DM; 3]; cyclophosphamide, 1 [SLE; 1]), 43 biologic disease-modifying antirheumatic drugs (bDMARDs) (etanercept, 16 [JIA, 12; DM, 2; DADA2, 2]; adalimumab, 10 [JIA, 10]; canakinumab, 8 [FMF, 7; CAPS, 1]; tocilizumab, 6 [JIA; 2; TA, 2; scleroderma, 2]; anakinra, 2 [FMF, 1; CAPS, 1]; rituximab, 1 [SLE, 1]); 21 systemic steroids (JIA, 10; SLE, 6; DM, 2; DADA2, 1; BD, 1; scleroderma, 1); and 6 patients were receiving acetyl-salicylic acid (SLE, 5; DADA2, 1) (Table 1). Four patients with ARF were under penicillin prophylaxis. Twenty-two patients with IRD excluding the ARF were in remission, and they were not receiving any treatment except non-steroidal anti-inflammatory drugs.

Before their vaccinations, 44 subjects recovered from COVID-19 (FMF, 18; JIA, 9; HC, 7; SLE, 5; ARF, 3; DM, 1; GPA, 1) (Table 1). While 4 of the recovered ones (HC, 2; JIA, 1; SLE, 1) had asymptomatic infection, the rest had mild COVID-19 symptoms. None of them had a severe clinical course.

While 214 subjects received BNT162b2 mRNA vaccine (FMF, 106; JIA, 49; HC, 19; SLE, 14; DM, 10; ARF, 4; CAPS, 2; scleroderma, 2; KD, 1; HSP, 1; BD, 1; DADA2, 1; Sjögren, 1; TA, 1; GPA, 1; BS, 1), 28 received inactivated SARS-CoV-2 vaccine (FMF, 16; JIA, 5; HC, 3; SLE, 2; DADA2, 1; scleroderma, 1), and 4 received both (FMF, 1; BD, 1; TA, 1; HC, 1) (Table 1).

Out of 246 subjects, 145 received a single dose of BNT162b2 mRNA vaccine, 19 received a single dose of inactivated SARS-CoV-2 vaccine, 69 received double doses of BNT162b2 mRNA vaccine, 8 received double doses of inactivated SARS-CoV-2 vaccine, 3 received double doses of inactivated SARS-CoV-2 vaccine plus a single dose of BNT162b2 mRNA vaccine, 1 received double doses of inactivated SARS-CoV-2 vaccine plus double doses of BNT162b2 mRNA vaccine, and 1 received 3 doses of inactivated SARS-CoV-2 vaccine.

3.2 | Adverse events

COVID-19 vaccine-related adverse events reported by the participants and their families were as follows: fatigue (n = 68, 27.6%), headache (n = 44, 17.9%), myalgia (n = 38, 15.4%), arthralgia (n = 38, 15.4%), fever (n = 35, 14.2%), nausea-vomiting (n = 19, 7.7%), diarrhea (n = 16, 6.5%), anorexia (n = 16, 6.5%), chest pain (n = 14, 5.7%), abdominal pain (n = 11, 4.5%), rhinorrhea (n = 8, 3.3%), arthritis (n = 8, 3.3%), cough (n = 8, 3.3%), dyspnea (n = 6, 2.4%), throat ache (n = 5, 2%), rash (n = 3, 1.2%), anosmia (n = 2, 0.8%), hypertension (n = 1, 0.4%), and hypotension (n = 1, 0.4%) (Figure 1).

Three subjects were considered to have severe adverse events, since they required hospitalization and additional treatment: 20.2 years-aged female patient with FMF who developed hypertension (2 weeks remained) after the second dose of BNT162b2 mRNA vaccine; 12.1 years-aged female with no underlying disease who experienced severe rash after the first dose of BNT162b2 mRNA vaccine; and 13.7 years-aged male patient with FMF who developed pre-syncope due to hypotension after the first dose of BNT162b2 mRNA vaccine.

All the adverse events but hypertension recovered in THE first 4 days. There was no adverse event after the administration of the second dose of CoronaVac inactive SARS-CoV-2 vaccine. Adverse event frequencies according to days and vaccine doses are given in Figure 2. Local reactions after the vaccines were seen in 20 subjects (JIA, 8; FMF, 7; HC, 3; DM, 1; BS, 1). Local reaction frequencies according to vaccine doses are also given in Figure 2.

Twenty-seven patients experienced disease flare within 1 month after the vaccination (after the first dose of BNT162b2 mRNA

TABLE 1 Baseline characteristics of the study population

	Healthy controls (n = 23)	Patients with AID (n = 126)	Patients with JIA (n = 54)	Patients with CTD (n = 30)	Patients with vasculitis (n = 9)	Patients with ARF (n = 4)
Age, y (median, min-max)	15.67 (12.04-19.94)	15.09 (12.06-20.72)	15.41 (12.06-20.64)	16.89 (12.49-20.64)	15.58 (12.02-20.92)	15.42 (13.71-18.1)
Gender						
Female, n (%)	10 (43.5%)	68 (54%)	35 (64.8%)	19 (63.3%)	6 (66.7%)	3 (75%)
Male, n (%)	13 (56.5%)	58 (46%)	19 (35.2%)	11 (36.7%)	3 (33.3%)	1 (25%)
Diagnoses (n)	-	FMF (123) CAPS (2) BS (1)	oJIA (43) jSPA (8) pJIA (3)	SLE (16) DM (10) Scleroderma (3) Sjögren (1)	BD (2) DADA2 (2) TA (2) GPA (1) HSP (1) KD (1)	-
Ongoing treatments						
Colchicine, n (%)	-	125 (99.2%)	-	-	3 (33.3%)	-
Steroid, n (%)	-	-	10 (18.5%)	9 (30%)	2 (22.2%)	-
ASA, n (%)	-	-	-	5 (16.7%)	1 (11.1%)	-
bDMARDs						
Anakinra, n (%)	-	2 (1.6%)	-	-	-	-
Canakinumab (n, %)	-	8 (6.3%)	-	-	-	-
Tocilizumab, n (%)	-	-	2 (3.7%)	2 (6.7%)	2 (22.2%)	-
Etanercept, n (%)	-	-	12 (22.2%)	2 (6.7%)	2 (22.2%)	-
Adalimumab, n (%)	-	-	10 (18.5%)	-	-	-
Rituximab, n (%)	-	-	-	1 (3.3%)	-	-
cDMARDs						
MTX, n (%)	-	-	12 (22.2%)	10 (33.3%)	-	-
Leflunomide, n (%)	-	-	9 (16.7%)	1 (3.3%)	-	-
Cyclosporine, n (%)	-	-	-	3 (10%)	-	-
Cyclophosphamide, n (%)	-	-	-	1 (3.3%)	-	-
HCQ, n (%)	-	-	-	21 (70%)	-	-
MMF, n (%)	-	-	-	6 (20%)	-	-
COVID-19 history before vaccination, n (%)	7 (30.4%)	18 (14.1%)	9 (17.3%)	6 (20%)	1 (11.1%)	3 (75%)
Vaccination info						
Vaccination type						
mRNA, n (%)	19 (82.6%)	109 (86.5%)	49 (90.7%)	27 (90%)	6 (66.7%)	4 (100%)

TABLE 1 (Continued)

	Healthy controls (n = 23)	Patients with AID (n = 126)	Patients with JIA (n = 54)	Patients with CTD (n = 30)	Patients with vasculitis (n = 9)	Patients with ARF (n = 4)
Inactive, n (%)	3 (13%)	16 (12.7%)	5 (9.3%)	3 (10%)	1 (11.1%)	-
Mix, n (%)	1 (4.3%)	1 (0.8%)	-	-	2 (22.2%)	-
Adverse events						
None, n (%)	12 (52.2%)	68 (54%)	33 (61.1%)	21 (70%)	3 (33.3%)	2 (50%)
Non-severe, n (%)	10 (435%)	56 (44.4%)	21 (38.9%)	9 (30%)	6 (66.7%)	2 (50%)
Severe, n (%)	1 (4.3%)	2 (1.6%)	-	-	-	-
Local reactions, n (%)	3 (13%)	8 (6.3%)	8 (14.8%)	1 (3.3%)	-	-
Disease flare within 1 month						
Yes, n (%)	-	15 (11.9%)	10 (18.5%)	2 (6.7%)	-	-
No, n (%)	-	111 (88.1%)	44 (81.5%)	28 (93.3%)	9 (100%)	4 (100%)

Abbreviations: AID, autoinflammatory diseases; ARF, acute rheumatic fever; ASA, acetylsalicylic acid; BD, Behçet disease; bDMARDs, biologic disease-modifying antirheumatic drugs; BS, Blau syndrome; CAPS, cryopyrin-associated periodic syndromes; cDMARDs, conventional disease-modifying antirheumatic drugs; CTD, connective tissue disease; DADA2, deficiency of adenosine deaminase 2; DM, dermatomyositis; FMF, familial Mediterranean fever; GPA, granulomatous polyangiitis; HCQ, hydroxychloroquine; HSP, Henoch-Schönlein purpura; JIA, juvenile idiopathic arthritis; jSPA, juvenile spondylarthritis; KD, Kawasaki disease; MMF, mycophenolate mofetil; MTX, methotrexate; oJIA, oligoarticular juvenile idiopathic arthritis; pJIA, polyarticular juvenile idiopathic arthritis; SLE, systemic lupus erythematosus; TA, Takayasu arteritis.

vaccine, 17; after the second dose of BNT162b2 mRNA vaccine, 7; after the first dose of CoronaVac inactive SARS-CoV-2 vaccine, 3) (FMF, 15; JIA, 10; SLE, 2). Among those who experienced disease flare, all patients with FMF presented with typical attacks (fever, abdominal pain, chest pain, and/or arthralgia), and all JIA patients developed new-onset arthritis. In addition to increased inflammatory markers, 1 of 2 patients with SLE had cutaneous involvement, and bicytopenia was seen in the other.

3.3 | Comparison of the participant groups

There were no significant differences between the HC group, biological group and non-biological group in terms of age, gender, vaccine types, and frequencies of pre-vaccination COVID-19 histories, local reactions and adverse events. Moreover, the frequency of disease flares within 1 month after vaccines was not different between the biological group and the non-biological group. Detailed data Are given in Table 2.

3.4 | Assessment of the risk factors for vaccine-related adverse events

There was no significant relationship between adverse event frequency and age, gender, the existing diseases, ongoing treatments (except acetylsalicylic acid [ASA]) and pre-vaccination COVID-19 histories. While the adverse event frequency was significantly lower in those who were receiving ASA during their vaccination period ($P = .037$), it was significantly higher in those who received the BNT162b2 mRNA vaccine ($P < .001$). Detailed data were given in Table 3.

4 | DISCUSSION

Out of 246 participants, 107 (43.5%) experienced COVID-19 vaccine-related adverse events in this study. Adverse events were seen after vaccine administration in 100 of 218 mRNA vaccines and 7 of 32 inactive vaccines. Since they required hospitalization, 2 patients with FMF under colchicine treatment and a healthy child were considered to have severe adverse events, and the remaining 104 were non-severe. All 3 occurred due to mRNA vaccines, and none of those with severe adverse events were under bDMARDs or cDMARDs treatment.

There was no significant differences between HC, non-biologic, and biologic groups with regard to the frequencies of vaccine-related adverse events and local reactions. However, the non-biologic group in the study was highly heterogeneous because it included patients in remission and patients receiving therapies that potentially alter the vaccine responses due to their B cell depletion effects, such as CYC or MMF.[16-18] Thus, sub-analyses were not possible in this study

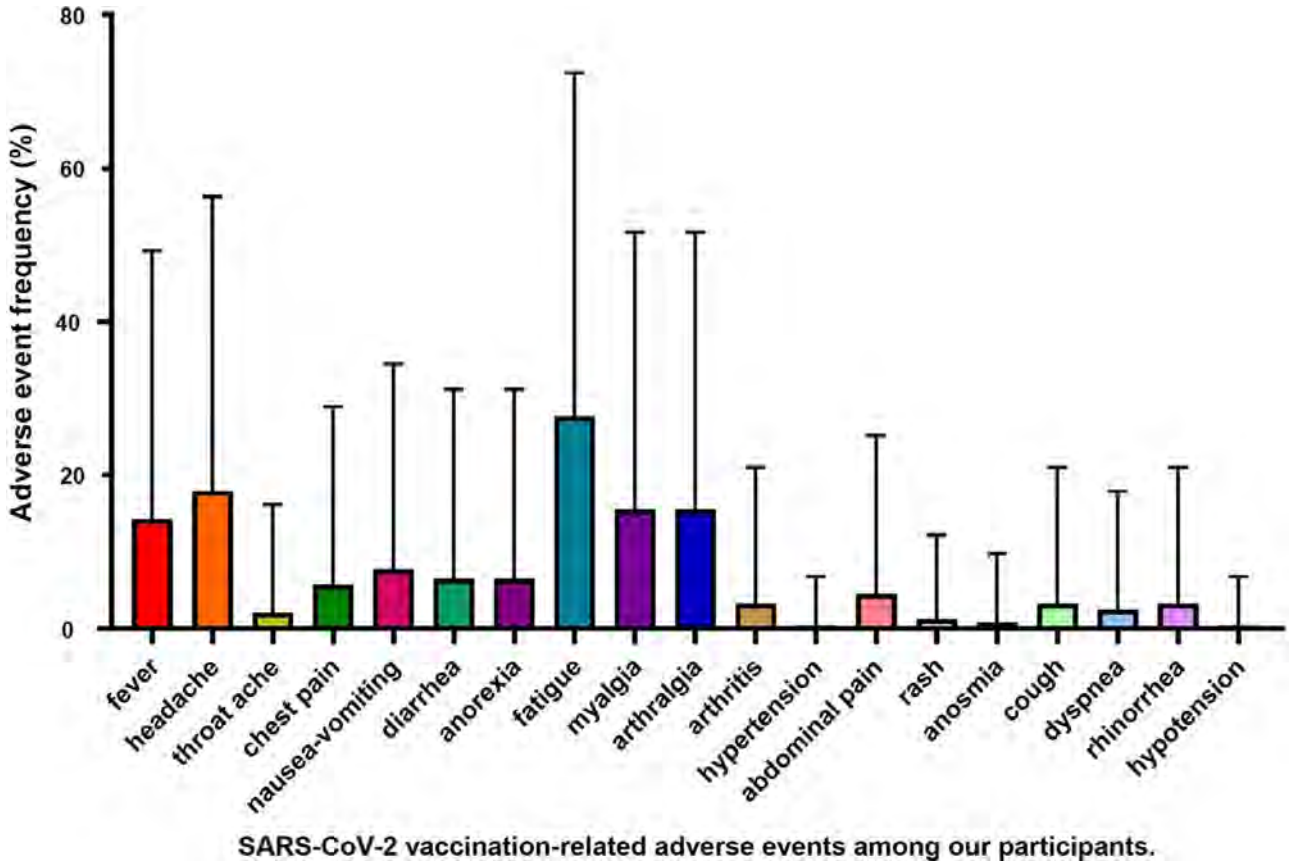


FIGURE 1 SARS-CoV-2 vaccination-related adverse events among our participants

While adverse events were significantly more common among the subjects who received the mRNA vaccine than those who received the inactive vaccine, there was no significant impact of age, gender, the existing diseases, ongoing treatments including DMARDs, and pre-vaccination COVID-19 histories on the adverse event frequency. The most common adverse events were fatigue, headache, myalgia, arthralgia, and fever, respectively. Local reactions were seen in 20 (8.13%) participants. Consistent with our findings, fatigue, headache, and muscle or joint pain were the most common vaccine-related systemic symptoms in the studies that enrolled adult patients with IRD.[19,20] Similarly, to the original phase 3 trial of the BNT162b2 COVID-19 mRNA vaccine, local pain in the injection site, fatigue and headache were the most common adverse events in a study that involved healthy adults and adult patients with SLE and rheumatoid arthritis. While reactogenicity was more frequent in the patient group, adverse events were not more severe than in the control group.[21]

Out of 27 (11%) patients who had disease flare within a 1-month period after the vaccines, those with JIA and MCTD required treatment modification, unlike 15 patients with FMF. Moreover, disease flare frequency was not different between biologic and non-biologic groups. Among the studies conducted in adult patients with IRD, while disease flare rate was 13.4% in the COVID-19 Global Alliance of Rheumatology Vaccine Study, it was reported as 5% in a study supported by the European League Against Rheumatism COVID-19 Vaccine Registry.[19,22] For accurate data regarding the disease flares, studies involving disease activity scores in all age groups are required.

Frequencies of local and systemic reactions caused by BNT162b2 COVID-19 mRNA vaccines were noted as 74% and 19%, respectively, in a recent study that involved 21 adolescents with JIA aged 16-21 years under anti-tumor necrosis factor (anti-TNF) treatment. Disease flares or serious adverse events were seen in none of the subjects. Although this study had a limited count of patients, it provided the first data on the vaccination of adolescent with IRD.[23] In our cohort, adverse events were seen in 10 of 26 patients under anti-TNF treatment and 21 of 54 patients with JIA, and similarly, none of them were serious.

In a phase 4 trial that evaluated immunogenicity and safety of the CoronaVac inactivated vaccine in adult patients with IRD, the most common systemic reactions were somnolence, headache, fatigue, and arthralgia, and none of them were moderate or severe. Systemic reaction frequencies after the first and second dose of the vaccine were 43.3%, and 33.4%, respectively.[24] Apart from local reactions, adverse events such as diarrhea, myalgia, arthritis, anosmia, anorexia, abdominal pain, rash, chest pain, and headache were seen in 7 of 32 CoronaVac inactivated vaccine administrations in our study. None of them remained for more than 2 days, and none of them were seen after the second dose. Consistent with the

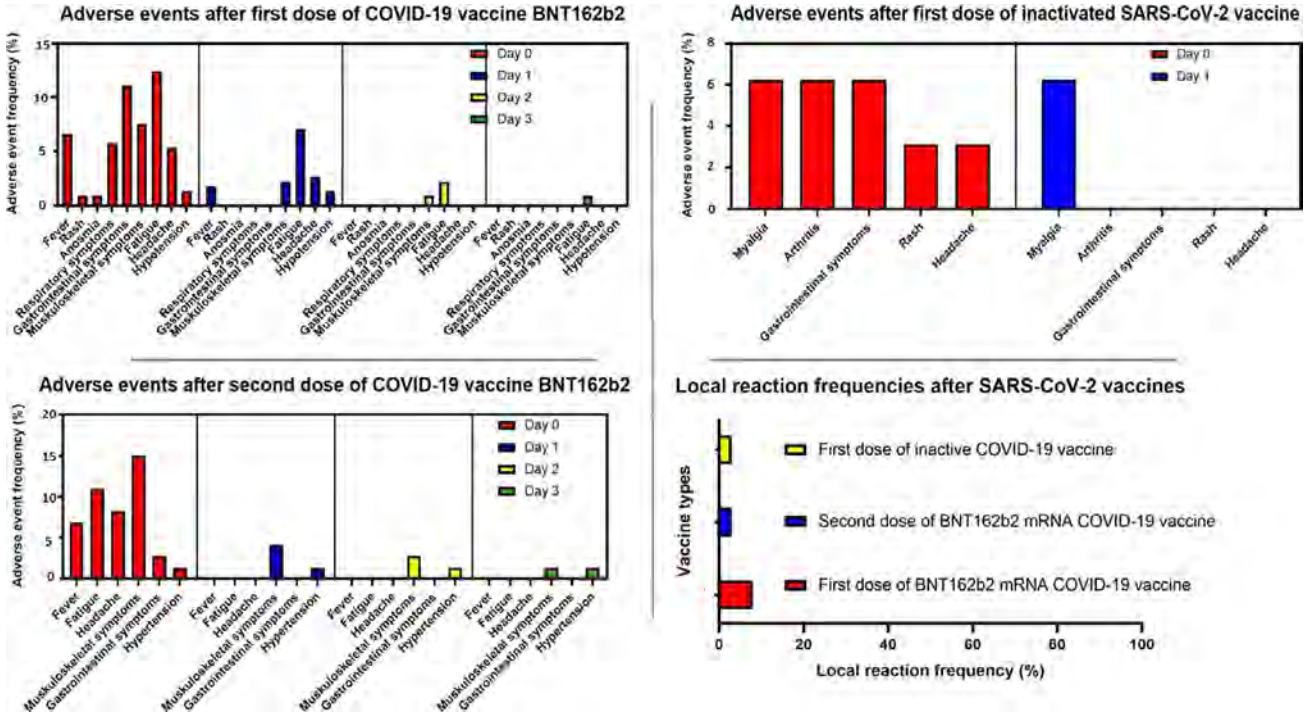


FIGURE 2 Adverse event frequencies according to days and vaccine types

TABLE 2 Comparison between the characteristics of healthy children, biologic group, and non-biologic group

	Healthy control group (n = 23)	Non-biologic group (n = 180)	Biologic group (n = 43)	*P*
Age, y (median, min-max)	15.67 (12.04-19.94)	15.14 (12.02-20.72)	16.09 (12.19-20.92)	.124
Gender				
Female, n (%)	10 (43.5%)	106 (58.9%)	25 (58.1%)	.369
Male, n (%)	13 (56.5%)	74 (41.1%)	18 (41.9%)	
Pre-vaccination COVID-19 history				
Yes, n (%)	7 (30.4%)	28 (15.6%)	9 (20.9%)	.182
No, n (%)	16 (69.6%)	152 (84.4%)	34 (79.1%)	
Vaccination type				
mRNA, n (%)	19 (82.6%)	160 (88.9%)	35 (81.4%)	.301
Inactive, n (%)	3 (13.0%)	18 (10.0%)	7 (16.3%)	
Mix, n (%)	1 (4.3%)	2 (1.1%)	1 (2.3%)	
Local reaction				
Yes, n (%)	3 (13.0%)	14 (7.8%)	3 (7.0%)	.581
No, n (%)	20 (87.0%)	166 (92.2%)	40 (93.0%)	
Disease flare within 1 month[a]				
Yes, n (%)	-	21 (11.7%)	6 (14.0%)	.680
No, n (%)	-	159 (88.3%)	37 (86.0%)	
Adverse events				
None, n (%)	12 (52.2%)	101 (56.1%)	26 (60.5%)	.579
Non-severe, n (%)	10 (43.5%)	77 (42.8%)	17 (39.5%)	
Severe, n (%)	1 (4.3%)	2 (1.1%)	0 (0.0%)	

[a]Healthy control group was not included into this analysis.

TABLE 3 Comparison of the patients with and without COVID-19 vaccine-related adverse events according to the baseline characteristics

	Adverse events		
	Yes (n = 107)	No (n = 139)	*P*
Age, y (median, min-max)	15.55 (12.02-20.92)	15.11 (12.18-20.72)	.376
Gender			
Female, n (%)	65 (60.7%)	76 (54.7%)	.340
Male, n (%)	42 (39.3%)	63 (45.3%)	
Disease			
Healthy control, n (%)	11 (10.3%)	12 (8.6%)	.323
Patients with AID, n (%)	58 (54.2%)	68 (48.9%)	
FMF, n	57	66	
CAPS, n	1	1	
BS, n	-	1	
Patients with JIA, n (%)	21 (19.6%)	33 (23.7%)	
oJIA, n	15	28	
jSPA, n	4	4	
pJIA, n	2	1	
Patients with CTD, n (%)	9 (8.4%)	21 (15.1%)	
SLE, n	4	12	
DM, n	4	6	
Scleroderma, n	1	2	
Sjögren, n	-	1	
Patients with vasculitis, n (%)	6 (5.6%)	3 (2.2%)	
BD, n	2	-	
DADA2, n	1	1	
TA, n	1	1	
GPA, n	1	-	
HSP, n	-	1	
KD, n	1	-	
Patients with ARF, n (%)	2 (1.9%)	2 (1.4%)	
Presence of a rheumatic disease, n (%)	96 (89.7%)	127 (%91.4)	.827
Ongoing treatments			
Colchicine, n (%)	60 (56.1%)	68 (48.9%)	.266
Steroid, n (%)	10 (9.3%)	11 (7.9%)	.819
ASA, n (%)	0 (0.0%)	6 (4.3%)	**.037**
bDMARDs, n (%)	17 (15.9%)	26 (18.7%)	.684
Anakinra, n	-	2	
Canakinumab, n	4	4	
Tocilizumab, n	3	3	
Etanercept, n	5	11	
Adalimumab, n	5	5	
Rituximab, n	-	1	
cDMARDs, n (%)[a]	18	31	
MTX, n	11	11	
Leflunomide, n	3	7	

TABLE 3 (Continued)

	Adverse events		
	Yes (n = 107)	No (n = 139)	*P*
Cyclosporine, n	3	-	
Cyclophosphamide, n	1	-	
HCQ, n	5	16	
MMF, n	3	3	
COVID-19 history before vaccination, n (%)			
Yes, n (%)	19 (17.8%)	25 (%18)	1
No, n (%)	88 (82.2%)	114 (%82)	
Vaccination type[b]			
mRNA, n	100	118	<.001
Inactive, n	7	25	

Abbreviations: AIDs, autoinflammatory diseases; ARF, acute rheumatic fever; ASA, acetylsalicylic acid; BD, Behçet disease; bDMARDs, biologic disease-modifying antirheumatic drugs; BS, Blau syndrome; CAPS, cryopyrin-associated periodic syndromes; cDMARDs, conventional disease-modifying antirheumatic drugs; CTD, connective tissue disease; DADA2, Deficiency of Adenosine Deaminase 2; DM, dermatomyositis; FMF, familial Mediterranean fever; GPA, granulomatous polyangiitis; HCQ, hydroxychloroquine; HSP, Henoch-Schönlein purpura; JIA, juvenile idiopathic arthritis; jSPA, juvenile spondylarthritis; KD, Kawasaki disease; MMF, mycophenolate mofetil; MTX, methotrexate; oJIA, oligoarticular juvenile idiopathic arthritis; pJIA, polyarticular juvenile idiopathic arthritis; SLE, systemic lupus erythematosus; TA, Takayasu arteritis.

[a]Total of cDMARDs rows are not equal to cDMARDs columns due to several patients being under poly-cDMARDs treatment.

[b]Four patients received both vaccination types; 3 experienced adverse events after mRNA vaccination, and 1 did not experience any adverse events.

previously mentioned phase 4 trial, none of them were considered serious. Although inactive vaccines are generally safe, there are concerns regarding the sufficient immunogenicity in patients with IRD, based on current findings.[25]

In order to achieve sufficient immunogenicity, although not contraindicated, the American College of Rheumatology (ACR) currently recommended withholding MTX, MMF and cyclophosphamide for 1-2 weeks following each COVID-19 dose in patients with well-controlled disease. This approach is mainly based on data from previous studies conducted with other vaccines, such as influenza and pneumococci.[14] However, findings of a recent study do not support temporarily cessation of MTX during vaccination in terms of seropositivity.[26] Due to the lack of data in the first days of the mass vaccination schedules and the concerns of the families regarding the disease activities, none of our patients discontinued their medication during the vaccination process. Adverse events per vaccine administration rates of the patients under treatment with MTX, MMF and cyclophosphamide were 11/22, 3/6, and 1/1, respectively. Although there was no safety issue in these patients because none of the adverse events were severe, further studies evaluating acceptable immunogenicity by measuring antibody levels are required.

Due to its B cell depletion effect, rituximab is another medical option that was recommended to be stopped during vaccination in the current ACR guidelines. It was proposed that, if the disease activities allow, the next rituximab cycle for patients must be delayed to 2-4 weeks after the final vaccine dose, to achieve acceptable antibody levels.[14] A recent study verified these suggestions by showing significantly impaired immunogenicity in patients receiving rituximab.[26] However, since both T cells and B cells have a pivotal role in the fight against SARS-CoV-2, it remains unclear whether vaccines may protect patients with an impaired humoral response.[27,28] Moreover, rituximab was shown to be significantly associated with severe COVID-19 disease course.[29]

In our cohort, there was only one patient under rituximab treatment during the vaccination period. He was a 16-year-old partially controlled SLE patient. In addition to rituximab, he was receiving MMF and HCQ. He had a COVID-19 infection history with mild to moderate symptoms before the vaccination. Therefore, he and his family had enormous concerns regarding re-infection with severe symptoms. He was vaccinated by double dose of CoronaVac inactivated vaccine based on his choice, and neither disease flares nor any adverse events were seen. Although he received his regular rituximab schedule with 1-month delay in line with current recommendations, we planned to examine him in terms of immunogenicity.

Vaccine hesitancy rapidly raised due to growing number of cases who developed vaccine-related severe or permanent adverse events such as myocarditis, hypertension, acute respiratory failure, septic shock, sudden hearing loss, and thromboembolic events.[30-33] Therefore, studies like ours that present a well-documented safety profile even in patients with IRD as a vulnerable group may ameliorate the concerns.

There are notable limitations in our study. First, dosages of immunosuppressive treatments of our patients are not available. Second, we did not assess the exact duration of the patients' medications and their disease activities. Third, given that the survey method was used as the first step for gathering data, selection bias may have occurred due to the possible willingness of the individuals who experienced adverse events for filling the questionnaire. Fourth, considering the

difficulty of sub-analyses due to a low number of patients, although CYC and MMF are known to potentially alter vaccine response, they were included in the non-biologic group. Although we did not assess the intervals between vaccination times and COVID-19 infection histories of the subjects, we know that our Ministry of Health regulations do not allow infected individuals to be vaccinated within the first 6 months. The main strength of the study is that this is the first one which evaluates adolescents and young adults with a broad spectrum of IRD in terms of vaccine-related adverse events.

In conclusion, our study indicates an acceptable safety profile of COVID-19 vaccines available in our country and encourages children with IRD to be vaccinated. Thus, prospective immunogenicity studies evaluating the efficacy of the vaccines and long-term follow-up studies for adverse events monitoring are required.

ACKNOWLEDGEMENTS

None

CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

All data relevant to the study are included in the article.

ORCID

Fatih Haslak https://orcid.org/0000-0002-6963-9668
Aybuke Gunalp https://orcid.org/0000-0003-0137-0460
Memnune Nur Cebi https://orcid.org/0000-0002-1327-0638
Mehmet Yildiz https://orcid.org/0000-0002-7834-4909
Amra Adrovic https://orcid.org/0000-0002-2400-6955
Sezgin Sahin https://orcid.org/0000-0002-5365-3457
Kenan Barut https://orcid.org/0000-0001-8459-2872
Ozgur Kasapcopur https://orcid.org/0000-0002-1125-7720

REFERENCES

1. Tanriover MD, Doğanay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *Lancet*. 2021;398(10296):213-222.
2. Thomas SJ, Moreira ED Jr, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine through 6 months. *N Engl J Med*. 2021;385(19):1761-1773.
3. Lu X, Zhang L, Du H, et al. SARS-CoV-2 infection in children. *N Engl J Med*. 2020;382(17):1663-1665.
4. Haslak F, Barut K, Durak C, et al. Clinical features and outcomes of 76 patients with COVID-19-related multi-system inflammatory syndrome in children. *Clin Rheumatol*. 2021;40(10):4167-4178.
5. Haslak F, Yıldız M, Adrovic A, Şahin S, Barut K, Kasapçopur Ö. A recently explored aspect of the iceberg named COVID-19: multisystem inflammatory syndrome in children (MIS-C). *Turk Arch Pediatr*. 2021;56(1):3-9.
6. Bachiller-Corral J, Boteanu A, Garcia-Villanueva MJ, et al. Risk of severe COVID-19 infection in patients with inflammatory rheumatic diseases. *J Rheumatol*. 2021;48(7):1098-1102.
7. Cordtz R, Lindhardsen J, Soussi BG, et al. Incidence and severeness of COVID-19 hospitalization in patients with inflammatory
8. Haslak F, Yildiz M, Adrovic A, et al. Management of childhood-onset autoinflammatory diseases during the COVID-19 pandemic. *Rheumatol Int*. 2020;40(9):1423-1431.
9. Yildiz M, Haslak F, Adrovic A, Sahin S, Barut K, Kasapcopur O. The frequency and clinical course of COVID-19 infection in children with juvenile idiopathic arthritis. *Clin Exp Rheumatol*. 2020;38(6):1271-1272.
10. Monti S, Balduzzi S, Delvino P, Bellis E, Quadrelli VS, Montecucco C. Clinical course of COVID-19 in a series of patients with chronic arthritis treated with immunosuppressive targeted therapies. *Ann Rheum Dis*. 2020;79(5):667-668.
11. Ouédraogo DD, Tiendrébéogo WJS, Kaboré F, Ntsiba H. COVID-19, chronic inflammatory rheumatic disease and anti-rheumatic treatments. *Clin Rheumatol*. 2020;39(7):2069-2075.
12. Hazlewood GS, Pardo JP, Barnabe C, et al. Canadian rheumatology association recommendation for the use of COVID-19 vaccination for patients with autoimmune rheumatic diseases. *J Rheumatol*. 2021;48(8):1330-1339.
13. Friedman MA, Curtis JR, Winthrop KL. Impact of disease-modifying antirheumatic drugs on vaccine immunogenicity in patients with inflammatory rheumatic and musculoskeletal diseases. *Ann Rheum Dis*. 2021;80(10):1255-1265.
14. Curtis JR, Johnson SR, Anthony DD, et al. American college of rheumatology guidance for COVID-19 vaccination in patients with rheumatic and musculoskeletal diseases: version 3. *Arthritis Rheumatol*. 2021;73(10):e60-e75.
15. Karayeva E, Kim HW, Bandy U, Clyne A, Marak TP. Monitoring vaccine adverse event reporting system (VAERS) reports related to COVID-19 vaccination efforts in Rhode Island. *R I Med J (2013)*. 2021;104(7):64-66.
16. Geisen UM, Berner DK, Tran F, et al. Immunogenicity and safety of anti-SARS-CoV-2 mRNA vaccines in patients with chronic inflammatory conditions and immunosuppressive therapy in a monocentric cohort. *Ann Rheum Dis*. 2021;80(10):1306-1311.
17. Izmirly PM, Kim MY, Samanovic M, et al. Evaluation of immune response and disease status in SLE patients following SARS-CoV-2 vaccination. *Arthritis Rheumatol*. 2021. doi:10.1002/art.41937
18. Zhu LP, Cupps TR, Whalen G, Fauci AS. Selective effects of cyclophosphamide therapy on activation, proliferation, and differentiation of human B cells. *J Clin Invest*. 1987;79(4):1082-1090.
19. Sattui SE, Liew JW, Kennedy K, et al. Early experience of COVID-19 vaccination in adults with systemic rheumatic diseases: results from the COVID-19 global rheumatology alliance vaccine survey. *RMD Open*. 2021;7(3):e001814.
20. Esquivel-Valerio JA, Skinner-Taylor CM, Moreno-Arquieta IA, et al. Adverse events of six COVID-19 vaccines in patients with autoimmune rheumatic diseases: a cross-sectional study. *Rheumatol Int*. 2021;41(12):2105-2108.
21. Bartels LE, Ammitzbøll C, Andersen JB, et al. Local and systemic reactogenicity of COVID-19 vaccine BNT162b2 in patients with systemic lupus erythematosus and rheumatoid arthritis. *Rheumatol Int*. 2021;41(11):1925-1931.
22. Machado P, Lawson-Tovey S, Hyrich K, et al. *LB0002 COVID-19 vaccine safety in patients with rheumatic and musculoskeletal disease*. BMJ Publishing Group Ltd; 2021.
23. Dimopoulou D, Spyridis N, Vartzelis G, Tsolia MN, Maritsi DN. Safety and tolerability of the COVID-19 mRNA-vaccine in adolescents with juvenile idiopathic arthritis on treatment with TNF-inhibitors. *Arthritis Rheumatol*. 2021. doi:10.1002/art.41977
24. Medeiros-Ribeiro AC, Aikawa NE, Saad CGS, et al. Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial. *Nat Med*. 2021;27(10):1744-1751.
25. Shenoy P, Ahmed S, Paul A, et al. Inactivated vaccines may not provide adequate protection in immunosuppressed patients with

26. Furer V, Eviatar T, Zisman D, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in adult patients with autoimmune inflammatory rheumatic diseases and in the general population: a multicentre study. *Ann Rheum Dis*. 2021;80(10):1330-1338.
27. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell*. 2020;181(7):1489-501.e15.
28. Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Xing Z. Immunological considerations for COVID-19 vaccine strategies. *Nat Rev Immunol*. 2020;20(10):615-632.
29. Strangfeld A, Schäfer M, Gianfrancesco MA, et al. Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 global rheumatology alliance physician-reported registry. *Ann Rheum Dis*. 2021;80(7):930-942.
30. Sessa M, Kragholm K, Hviid A, Andersen M. Thromboembolic events in younger women exposed to Pfizer-BioNTech or moderna COVID-19 vaccines. *Expert Opin Drug Saf*. 2021;20(11):1451-1453.
31. Jeong J, Choi HS. Sudden sensorineural hearing loss after COVID-19 vaccination. *Int J Infect Dis*. 2021;113:341-343.
32. Kewan T, Flores M, Mushtaq K, et al. Characteristics and outcomes of adverse events after COVID-19 vaccination. *J Am Coll Emerg Physicians Open*. 2021;2(5):e12565.
33. King WW, Petersen MR, Matar RM, Budweg JB, Cuervo Pardo L, Petersen JW. Myocarditis following mRNA vaccination against SARS-CoV-2, a case series. *Am Heart J plus*. 2021;8:100042.

How to cite this article: Haslak F, Gunalp A, Cebi MN, et al. Early experience of COVID-19 vaccine-related adverse events among adolescents and young adults with rheumatic diseases: A single-center study. *Int J Rheum Dis*. 2022;00:1–11. doi:10.1111/1756-185X.14279

6.10. Estudo mostra que CoronaVac é segura e imunogênica para crianças com idades entre sete meses e cinco anos

Um estudo de vacinação com a CoronaVac realizado por cientistas do Instituto Adolfo Lutz, do Instituto de Infectologia Emílio Ribas e da Secretaria de Estado da Saúde de São Paulo concluiu que a CoronaVac é segura e imunogênica para crianças. A pesquisa foi realizada com 27 brasileiros, com idades entre sete meses e cinco anos, que receberam a vacina do Butantan e da farmacêutica chinesa Sinovac de modo inadvertido nas cidades de Diadema e Itirapina, no estado de São Paulo. Apenas uma delas apresentou sintomas leves, sem outros eventos adversos importantes registrados durante o acompanhamento de 30 dias.

As crianças participantes do estudo buscaram unidades básicas de saúde (UBS) para tomar a vacina da influenza, mas acabaram recebendo por engano a CoronaVac. O evento foi imediatamente comunicado às secretarias de saúde de cada município e, em relação ao evento adverso, ao sistema de vigilância vacinal. O Centro de Vigilância Epidemiológica da Secretaria de Estado da Saúde de São Paulo (CVE) e o Instituto Adolfo Lutz atenderam as secretarias de Itirapina e Diadema.

As 27 crianças vacinadas com uma única dose foram monitoradas por pediatras, que coletaram amostras de soro na primeira consulta (nove dias após a vacinação) e após 30 dias da imunização. A única criança que relatou efeitos adversos tinha dois anos e apresentou coriza na primeira consulta após a vacinação.

Todas as crianças foram testadas para sorologia SARS-CoV-2 S1 com proteína Ortho IgG anti-S1 total e Cpass, um método que permite a rápida detecção de anticorpos neutralizantes totais. Cinco delas tinham título de proteína IgG total superior a 1.0 (testes de reagentes) entre três e nove dias após a vacinação. Do total, 19 tiveram o sangue coletado 30 dias depois da aplicação e também apresentaram títulos totais de proteína IgG spike superior a 1.0. Quatro das cinco crianças que apresentaram teste reagente na primeira consulta foram testadas novamente um mês depois da imunização e apresentaram aumento da proteína spike IgG anti S1 total, passando de uma média de 10,4 para 20,5.

Os objetivos do estudo eram descrever a resposta da saúde pública a um erro programático e monitorar a segurança, tolerabilidade e soroconversão da vacina por meio da detecção da quantidade total de anticorpos IgG contra a proteína spike SARS-CoV-2 S1 após a vacinação de crianças com CoronaVac.

Publicado em: 6/12/2021

REVISTA DO INSTITUTO DE MEDICINA TROPICAL DE SÃO PAULO

JOURNAL OF THE SÃO PAULO INSTITUTE OF TROPICAL MEDICINE

BRIEF COMMUNICATION

http://doi.org/10.1590/S1678-9946202163083

Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in inadvertently vaccinated healthy children

Eder Gatti Fernandes [1,2], Giselle Ibette Silva López-Lopes [3], Valeria Oliveira Silva [3], Rosemeire Yamashiro [3], Karen Cristina Rolim Madureira [2], Juliana Failde Gallo [2], José Angelo Lindoso [2,4,5], Helena Keico Sato [1], Núbia Virginia D'Avila Limeira de Araujo [1], Maria Ligia Bacciotte Ramos Nerger [1], Luis Fernando Macedo Brigido [6]

[1]Secretaria de Estado da Saúde de São Paulo, Coordenadoria de Controle de Doenças, Centro de Vigilância Epidemiológica "Prof. Alexandre Vranjac", Divisão de Imunização, São Paulo, São Paulo, Brazil

[2]Instituto de Infectologia Emílio Ribas, São Paulo, São Paulo, Brazil

[3]Instituto Adolfo Lutz, Centro de Imunologia, São Paulo, São Paulo, Brazil

[4]Universidade de São Paulo, Faculdade de Medicina, Departamento de Moléstias Infecciosas e Parasitárias, São Paulo, São Paulo, Brazil

[5]Universidade de São Paulo, Instituto de Medicina Tropical de São Paulo, São Paulo, São Paulo, Brazil

[6]Instituto Adolfo Lutz, Centro de Virologia, São Paulo, São Paulo, Brazil

Correspondence to: Eder Gatti Fernandes, Secretaria de Estado da Saúde de São Paulo, Coordenadoria de Controle de Doenças, Centro de Vigilância Epidemiológica "Prof. Alexandre Vranjac", Divisão de Imunização, Av. Dr Arnaldo, 351, 6º andar, Pacaembu, CEP 01246-000 São Paulo, SP, Brazil
Tel: +55 11 3066-8779

E-mail: edergatti@saude.sp.gov.br

Received: 13 August 2021

Accepted: 4 October 2021

ABSTRACT

Twenty-seven children aged seven months to 5 years were inadvertently vaccinated with a COVID-19 vaccine, the CoronaVac (Sinovac, China), an inactivated SARS-CoV-2 vaccine, in two different cities of Sao Paulo State, Brazil. After the event, these children were monitored by local pediatricians and serum samples were collected at the first visit and 30 days after vaccination and tested for SARS-CoV-2 S1 serology with Ortho total IgG anti-S1 protein and Cpass, an ACE2 receptor binding domain inhibition assay. Only one child had a mild symptom after vaccination, with no other adverse events documented up to the 30 days follow-up. Of 27 children tested 3-9 days after vaccination, 5 (19%) had positive serology suggesting a previous natural SARS-CoV-2 infection, with all 19 tested on day 30 after vaccination and presenting with positive tests, with an increment of antibody titers in those initially positive. A low Cpass binding inhibition was observed in the first collection in 11 seronegative cases, with high titers among those anti-S1 positive. All children showed an important increase in antibody titers on day 30. The event allowed the documentation of a robust serological response to one dose of CoronaVac in this small population of young children, with no major adverse effects. Although it was an unfortunate accident, this event may contribute with future vaccine strategies in this age group. The data suggest that CoronaVac is safe and immunogenic for children.

KEYWORDS: COVID-19 vaccines. Adverse events. Brazil.

INTRODUCTION

On May 22nd, 2021, 27 healthy children were inadvertently vaccinated with a COVID-19 vaccine CoronaVac, instead of receiving the influenza vaccine in a primary health care unit in Itirapina, a small city in the countryside of Sao Paulo State, Brazil. One day later (May the 23rd), the same error happened in Diadema, a city located in the metropolitan area of Sao Paulo city, where five children were also inadvertently vaccinated with CoronaVac.

CoronaVac is an inactivated SARS-CoV-2 vaccine developed by Sinovac Life Sciences (Beijing, China), which has been used among adults aged ≥18 years in Brazil, since January 2021. This vaccine is produced by Sinovac in partnership with the local public vaccine manufacturer Butantan[1]. Over 40 million doses of CoronaVac had already been administered by the end of June 2021 all over the country[2].

The vaccination error was promptly reported to the ealth department of each municipality and, in relation to dverse events, to the vaccination surveillance system. The pidemiological Surveillance Center of Sao Paulo State CVE) and the Adolfo Lutz Institute assisted the health epartments of Itirapina and Diadema. The objectives were o describe the public heath response to a programmatic ror and to monitor the vaccine safety, tolerability and eroconversion by detecting the total amount of IgG ntibodies against SARS-CoV-2 S1 spike protein after the accination of children with CoronaVac.

IATERIALS AND METHODS

The children who had been inadvertently vaccinated ith CoronaVac (Sinovac Life Sciences, Beijing, China) ere monitored by pediatricians in primary health care nits for 30 days, to receive medical assistance if any sign or mptom appeared. Reports of their health conditions were nt to the health department of each municipality. Three isits were scheduled for medical evaluation, right after the vent recognition (error in the vaccine used), at 15th and 30th ay after vaccination. To inform the families and local health orkers caring for these children of their serological status, vo registered assays, available at State public laboratories ere used. Blood samples were taken on the first medical valuation (3-9 days after the event) and on the 30th day ter the vaccination event. The presence of antibodies for ARS-CoV-2 were detected using (i) a chemiluminescent icroparticle assay (VITROS® Anti-SARS-CoV2, Ortho linical Diagnostics, United Kingdom) which detects the omain of the S1 (spike) antigen, considering sororeactive r SARS-CoV-2 antibodies samples with titers >1.0 and; i) the evaluation of antibodies able to interfere with the BD-ACE2 interaction (RBI), measured by cPass (SARS-oV-2 Neutralization Antibody Detection kit, GenScript, SA), both test performed following the manufacturer's nstructions. The test was considered positive for the resence of neutralizing antibodies for SARS-CoV-2 when n inhibition titer ≥ 20% is obtained, and samples are ssigned as presenting with low inhibition when percentages om 5% to 20% inhibition are detected.

All clinical information and laboratory tests results were gistered in each case, reporting the clinical manifestations f adverse events to the health departments and to the rogrammatic error surveillance system.

The approach to these children occurred only after the etection of the error in the type of vaccine used, when their blood samples were collected to perform the serological assays. Those that agreed to participate in the serological evaluation were oriented to return after 30 days after vaccination for retesting. The present investigation was the official response to a public health crisis, thus it did not require the approval of an ethical council.

RESULTS

Table 1 shows the characteristics of CoronaVac vaccinated children. From the total of 27 children, 52% were male, with ages ranging from 7 months to 5 years. Only one 2-years-old child presented a symptom (running nose) during the first visit, nine days after vaccination. No other symptoms were reported among the infants in the 30 days following the vaccination.

All children (n=27) were tested at the first visit for S1 antibodies and 5 (18.5%) had total S1 spike protein IgG titer higher than 1.0 (reagent tests) 3-9 days after vaccination. Nineteen had blood collected 30 days after vaccination and all of them had total S1 spike protein IgG titers higher than 1.0 (reagent tests). Four of the five children who presented reagent tests at the first visit were retested on the 30th day after vaccination, all showing an increased total IgG anti S1 spike protein, going from a mean of 10.4 to a mean value of 20.5. About half (47%, 9/19) tested for the receptor binding domain inhibition (RBI) showed results above 20%, but most had a low binding inhibition (5-20%), with only three cases, all S1 seropositive, with high titers (over 90% inhibition). On the 30th day, 12/13 tested children had titers above 30%, with a median titer of 45% (IQR 36-65). Titers of S1 have also increased from the initial collection up to the 30th day, from 0.1 (IQR 0-0.3) to 7.9 (5.5-11.2).

DISCUSSION

No COVID-19 vaccines are authorized in Brazil, so far, for use in children under the age of 12 years. However, a phase 2 study has already assessed the safety, tolerability and immunogenicity of CoronaVac in the population aged 3 to 17 years[3].

We presented a response to a programmatic error situation. Despite the vaccination error, all monitored children did not show adverse events following the immunization. The analyses from phase 1–3 trials have shown that CoronaVac was safe in adults aged 18 years and older[4]. A Phase 1-2 study evaluated children and adolescents

able 1 - Demographic and serological results from children inadvertently vaccinated with CoronaVAc (one dose), Sao Paulo State, razil, 2021.

ex	Age (months)	DV 1	DV 2	S1 Ab 1	S1 Ab 2	RBI 1	RBI 2
emale	22	4	NA	0.01	NA	5.00	NA
emale	28	4	30	0.00	6.49	19.61	30.95
emale	42	4	30	3.11	19.00	39.90	NA
emale	69	4	NA	0.01	NA	NA	NA
emale	44	4	30	0.00	7.53	-6.89	45.22
emale	30	4	NA	11.30	NA	NA	NA
emale	3	6	30	0.01	7.73	9.07	62.34
emale	60	7	NA	0.01	NA	NA	NA
emale	7	3	33	0.00	10.10	21.83	64.87
emale	37	3	33	0.00	3.03	3.60	33.04
emale	60	3	33	0.00	7.94	8.73	51.00
emale	54	9	NA	0.02	NA	NA	NA
ale	52	4	NA	0.01	NA	-0.69	NA
ale	31	4	NA	0.00	NA	NA	NA
ale	23	4	30	0.00	3.77	NA	22.05
ale	22	4	NA	0.03	NA	NA	NA
ale	60	4	30	5.17	20.50	91.50	96.8
ale	31	4	30	0.00	3.00	27.12	35.84
ale	46	4	30	0,.00	10.20	-10.54	38.68
ale	10	4	30	0.00	8.90	22.99	68.12
ale	13	4	30	0.00	11.20	22.50	68.96
ale	49	4	30	0.01	4.19	13.21	35.79
ale	35	4	30	0.03	5.48	23.48	38.06
ale	32	4	41	0.01	9.73	NA	NA
ale	18	3	33	19.00	24.10	97.07	NA
ale	54	5	34	0.17	6.95	19.48	57.98
ale	23	9	30	13.30	18.60	97.36	NA

V 1 = days after the 1st dose of vaccine and first blood sampling ; DV 2 = days after the 1st dose of vaccine and 2nd blood sampling; 1 Ab 1= antibody titers against the SPIKE domain S1 at the time of the 1st blood sampling ; S1 Ab 2 = antibody tites against the PIKE domain S1 at the time of the 2nd blood sampling ; RBI 1 = percentage of receptor binding inhibition at the time of the 1st blood ampling ; RBI 2 = percentage of receptor binding inhibition at the time of the 2st blood sampling ; NA = not available.

vents were non-severe, and the most common reactions ere pain at the injection site and fever[3].

All tested children showed an increase in total S1 spike rotein IgG antibodies 30 days following the vaccination. lthough some children already had antibodies at the time f the initial blood collection, presumably due to previous symptomatic, unrecognized infection by SARS-CoV-2. hen these previously positive children were tested 30 days ter the vaccination, they showed an increment in IgG inding antibody units at the second blood sampling. As no ıfection during the observation period was documented, inhibition, a functional assay to evaluate the ability of serum samples to interfere with the binding of the viral receptor binding domain of the S1 protein with the cellular receptor ACE-2, showed some inhibition (from 5 to 20%) in 11 children that did not had total anti S1 IgG antibodies[5]. The titers were however low and may represent either unspecific reactivity or a previous exposure to other coronaviruses. The limited information of the test in particular in this age group, does not allow us to come to any conclusion, but all retested children on the 30th day after vaccination showed important increments in RBI titers, with only one case below

mited to a serological response to S1 antigens, either otal IgG to the viral S1 protein binding inhibition to the ajor SARS-CoV-2 receptor, the data suggest an anti-spike sponse after one dose of the vaccine. In other words, one ose of CoronaVac was immunogenic in children[3].

Wrong vaccine administration is the most reported accination error[7,8]. CoronaVac and influenza vaccines used the Brazilian public health system come from the same cal producer (Butantan) and they have the multiple dose esentation, which could favor the confusion. However, ie label and the color of the bottle cap are different. The urrent high number of different vaccines available in the razilian immunization schedule demands well trained ealth professionals. Vaccination errors may harm patients nd cause a negative impact on the population's confidence n vaccination, which in turn will negatively impact the accination coverage[8].

This study has some limitations. Firstly, it is a response an unexpected event, justifying the small sample size that oes not allow us to rule out the occurrence of rare adverse vents or even to definitely conclude on the duration of ie seroconversion observed after the first dose. Secondly, nildren did not receive the second dose and were not valuated after the end of the proposed immunization. hirdly, the cellular immunity was not evaluated. Finally, ie monitoring period (30 days) was short to determine ng-term immunogenicity and also for a complete valuation of safety.

Children infected with SARS-CoV-2 mainly have ild disease or are asymptomatic, when compared with dults. However, a small number of children, especially ose with health comorbidities, might be at risk of severe OVID-19[9,10]. Furthermore, the SARS-CoV-2 infection an lead to a serious, although rare complication called ie multisystem inflammatory syndrome in children[11]. inally, children can be transmitters of SARS-CoV-2 in mmunities[12]. A vaccine against SARS-CoV-2 for children nd adolescents will contribute decisively to the control f the COVID-19 pandemic. Our investigation suggests at CoronaVac is well tolerated and safe and can induced umoral responses in children, but proper safety and ffectiveness studies must be performed before expanding ie vaccination to young children.

CKNOWLEDGMENTS

The authors would like to thank all the collaborators om the Health Departments of Diadema and Itirapina, the

AUTHORS' CONTRIBUTIONS

EGF, HKS, NVDLA, MLBRN, and LFMB conducted the investigation together with the technicians of the municipality of Diadema and Itirapina; GISL, VOS, RY, KCRM, JFG, JAL, and LFMB performed the laboratory assay; EGF drafted the initial manuscript. GISL, HKS, NVDLA, and LFMB reviewed the manuscript. All authors approved the final manuscript as submitted.

CONFLICT OF INTERESTS

None.

FUNDING

None.

REFERENCES

1. São Paulo. Centro de Vigilância Epidemiológica "Prof. Alexandre Vranjac". Divisão de Imunização. Documento técnico: campanha de vacinação contra a COVID-19: 23 de julho de 2021, 18ª atualização. [cited 2021 Nov 5]. Available from: https://portal.saude.sp.gov.br/resources/cve-centro-de-vigilancia-epidemiologica/vacina/documentos-tecnicos-covid-19/documentotecnico_campanhadevacinacaocontraacovid_18atualizacao_23_07.pdf
2. Brasil. Ministério da Saúde. LocalizaSUS. [cited 2021 Nov 5]. Available from: https://localizasus.saude.gov.br/
3. Han B, Song Y, Li C, Yang W, Ma Q, Jiang Z, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021 In Press.
4. Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021;21:181-92.
5. Taylor SC, Hurst B, Charlton CL, Bailey A, Kanji JN, McCarthy MK, et al. A new SARS-CoV-2 dual-purpose serology test: highly accurate infection tracing and neutralizing antibody response detection. J Clin Microbiol. 2021;59:e02438-20.
6. Tan SS, Saw S, Chew KL, Huak CY, Khoo C, Pajarillaga A, et al. Head-to-head evaluation on diagnostic accuracies of six SARS-CoV-2 serological assays. Pathology. 2020;52:770-7.

3. Braga PC, Silva AE, Mochizuki LB, Lima JC, Sousa MR, Bezerra AL. Incidence of post-vaccination adverse events in children. J Nurs UFPE Online. 2017;11 Suppl 10:4126-35.
9. Zimmermann P, Curtis N. Coronavirus infections in children including COVID-19: an overview of the epidemiology, clinical features, diagnosis, treatment and prevention options in children. Pediatr Infect Dis J. 2020;39:355-68.
10. Maltezou HC, Magaziotou I, Dedoukou X, Eleftheriou E, Raftopoulos V, Michos A, et al. Children and adolescents with SARS-CoV-2 infection: epidemiology, clinical course and viral loads. Pediatr Infect Dis J. 2020;39:e388-92.
11. Ebina-Shibuya R, Namkoong H, Shibuya Y, Horita N. Multisystem inflammatory syndrome in children (MIS-C) with COVID-19: insights from simultaneous familial Kawasaki disease cases. Int J Infect Dis. 2020;97:371-3.
12. Kao CM, Orenstein WA, Anderson EJ. The importance of advancing severe acute respiratory syndrome coronavirus 2 vaccines in children. Clin Infect Dis. 2021;72:515-8.

6.11. Revisão sistemática de estudos científicos atesta segurança e eficácia da CoronaVac para crianças e adolescentes

Pesquisadores chineses realizaram uma revisão sistemática sobre estudos controlados e randomizados, estudos de caso e seriados com o objetivo de estimar a segurança, imunogenicidade e eficácia da vacinação de crianças e adolescentes contra a Covid-19. A pesquisa foi conduzida por cientistas da Universidade Médica de Chongqing, da Universidade de Lanzhou e do Centro Nacional de Pesquisa Médica sobre Saúde e Doenças Infantis da China e publicado no periódico Vaccines em meados de setembro de 2021.

Os pesquisadores investigaram estudos publicados até 23/7/2021 nas plataformas PubMed, Web of Science, no database sobre Covid-19 da Organização Mundial da Saúde (OMS) e no Instituto Nacional da China para Infraestrutura do Conhecimento (CNKI, na sigla em inglês).

Foram incluídos na revisão oito estudos publicados, envolvendo um total de 2.852 crianças, e 28 estudos clínicos em andamento. Uma das principais pesquisas analisadas foi o ensaio clínico randomizado controlado de fase 1 e 2 do uso da CoronaVac entre crianças de três a 17 anos realizado na China. Os demais papers são referentes a vacina desenvolvida com a tecnologia de RNA mensageiro.

Segundo a revisão, o ensaio clínico da CoronaVac mostrou que a vacina tem bom perfil de segurança e é imunogênica para crianças e adolescentes. Em relação à segurança, a maioria dos eventos adversos foi leve ou moderado, como dor no local da injeção, fadiga, dor de cabeça e dor no peito. Quanto à imunogenicidade, tanto na fase 1 quanto na fase 2, a soroconversão de anticorpos neutralizantes após a segunda dose foi de 100%.

"Nossa revisão encontrou altos níveis de imunogenicidade e eficácia vacinal em crianças e adolescentes. Esse é um claro indicador de que as vacinas são efetivas, e os estudos controlados randomizados também não se depararam com grandes questões em relação a segurança", concluem os pesquisadores.

A vacina é a forma mais eficaz de prevenir e controlar infecções por Covid-19, além de estimular o sistema imunológico a produzir anticorpos. Promover a vacinação de crianças e adolescentes é crucial para barrar a propagação do coronavírus, já que esse grupo representa um quarto da população mundial.

Publicado em: 29/9/2021

Artigo

vaccines

MDPI

Systematic Review

Safety, Immunogenicity, and Efficacy of COVID-19 Vaccines in Children and Adolescents: A Systematic Review

Meng Lv [1,2,3,†], Xufei Luo [4,†], Quan Shen [2,3,5], Ruobing Lei [2,3,5], Xiao Liu [4], Enmei Liu [2,3,6], Qiu Li [1,2,3,*] and Yaolong Chen [7,8,9,10,11,12,*]

1 Department of Nephrology, Children's Hospital of Chongqing Medical University, Chongqing 400014, China; lvm2016@163.com
2 National Clinical Research Center for Child Health and Disorders, Ministry of Education Key Laboratory of Child Development and Disorders, China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Children's Hospital of Chongqing Medical University, Chongqing 400014, China; shenquan@whu.edu.cn (Q.S.); leiruobing1009@163.com (R.L.); emliu186@126.com (E.L.)
3 Chongqing Key Laboratory of Pediatrics, Chongqing 400014, China
4 School of Public Health, Lanzhou University, Lanzhou 730000, China; luoxf2016@163.com (X.L.); liuxiao20@lzu.edu.cn (X.L.)
5 Chevidence Lab Child & Adolescent Health, Department of Pediatric Research Institute, Children's Hospital of Chongqing Medical University, Chongqing 400014, China
6 Department of Respiratory Medicine, Children's Hospital of Chongqing Medical University, Chongqing 400014, China
7 Institute of Health Data Science, Lanzhou University, Lanzhou 730000, China
8 Evidence-Based Medicine Center, School of Basic Medical Sciences, Lanzhou University, Lanzhou 730000, China
9 WHO Collaborating Centre for Guideline Implementation and Knowledge Translation, Lanzhou 730000, China
10 Guideline International Network Asia, Lanzhou 730000, China
11 Key Laboratory of Evidence Based Medicine and Knowledge Translation of Gansu Province, Lanzhou University, Lanzhou 730000, China
12 Lanzhou University GRADE Center, Lanzhou 730000, China
* Correspondence: liqiu21@126.com (Q.L.); chenyaolong@vip.163.com (Y.C.)
† These authors contributed equally to this work.

check for updates

Citation: Lv, M.; Luo, X.; Shen, Q.; Lei, R.; Liu, X.; Liu, E.; Li, Q.; Chen, Y. Safety, Immunogenicity, and Efficacy of COVID-19 Vaccines in Children and Adolescents: A Systematic Review. *Vaccines* **2021**, *9*, 1102. https://doi.org/10.3390/vaccines9101102

Academic Editor: François Meurens

Received: 1 August 2021
Accepted: 24 September 2021
Published: 29 September 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Aim: To identify the safety, immunogenicity, and protective efficacy of COVID-19 vaccines in children and adolescents. Methods: We conducted a systematic review of published studies and ongoing clinical studies related to the safety, immunogenicity, and efficacy of COVID-19 vaccine in children or adolescents (aged < 18 years). Databases including PubMed, Web of Science, WHO COVID-19 database, and China National Knowledge Infrastructure (CNKI) were searched on 23 July 2021. International Clinical Trials Registry Platform (ICTRP) was also searched to identify ongoing studies. Results: Eight published studies with a total of 2852 children and adolescents and 28 ongoing clinical studies were included. Of the eight published studies, two were RCTs, two case series, and four case reports. The investigated COVID-19 vaccines had good safety profiles in children and adolescents. Injection site pain, fatigue, headache, and chest pain were the most common adverse events. A limited number of cases of myocarditis and pericarditis were reported. The RCTs showed that the immune response to BNT162b2 in adolescents aged 12–15 years was non-inferior to that in young people aged 16–25 years, while with 3 μg CoronaVac injection the immune response was stronger than with 1.5 μg. The efficacy of BNT162b2 was 100% (95% CI: 75.3 to 100), based on one RCT. Of the 28 ongoing clinical studies, twenty-three were interventional studies. The interventional studies were being conducted in fifteen countries, among them, China (10, 43.5%) and United States(9, 39.1%) had the highest number of ongoing trials. BNT162b2 was the most commonly studied vaccine in the ongoing trials. Conclusion: Two COVID-19 vaccines have potential protective effects in children and adolescents, but awareness is needed to monitor possible adverse effects after injection. Clinical studies of the COVID-19 vaccination in children and adolescents with longer follow-up time, larger sample size, and a greater variety of vaccines are still urgently needed.

Keywords: COVID-19; vaccine; children; adolescents; systematic review

1. Background

One and a half year have passed since the beginning of the coronavirus disease 2019 (COVID-19) pandemic. Yet the epidemic is still not under control. With over 200 million confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections and over 4 million COVID-19 related deaths, COVID-19 has brought great suffering and devastation to people worldwide.

Vaccines, as an effective way to prevent and control disease infections, stimulate the human immune system to produce antibodies, thus increasing immunity to the disease and generating protection for the immunized individual [1]. Vaccination aims to curb the spread of the disease and helps to potentially achieve herd immunity. As of 18 September 2021, twenty-two COVID-19 vaccines worldwide have been approved [1]. However, we have little knowledge of the efficacy and safety of COVID-19 vaccines in children and adolescents. Given that children and adolescents account for approximately one quarter of the world's population [2], promoting vaccination of children and adolescents is also crucial to end the spread of COVID-19.

The development of COVID-19 vaccine has been in full swing since the COVID-19 outbreak. Studies have shown that the current COVID-19 vaccines are effective and safe in adults [3–6]. Several international organizations and countries have also developed guidelines for different aspects of COVID-19 vaccination, including vaccination of special populations, management of adverse reactions, and cautions for vaccination [7–9]. However, the efficacy of protection and adverse effects of COVID-19 vaccines in children and adolescents remains unclear despite a large number of clinical trials being conducted. Furthermore, children and adolescents have less severe COVID-19 symptoms than adults [10], and they likely play a limited role in spreading the infection to others. Therefore, more high-quality clinical studies are still needed to determine whether COVID-19 vaccination should be recommended for children at the moment [11]. In addition, children are a population group with special needs and features, and the attitude of parents or guardians toward the COVID-19 vaccine is also an essential factor affecting children's vaccination. To explore and promote COVID-19 vaccination in children and adolescents, The National Clinical Research Center for Child Health and Disorders (Chongqing, China) initiated an international guideline for the management of COVID-19 in children and adolescents [12] that also contains the question of whether and how children and adolescents should be vaccinated against COVID-19. To answer this question, we conducted a systematic review to estimate the safety, immunogenicity, and protective efficacy of the COVID-19 vaccine in children and adolescents, covering both completed and ongoing studies and trials.

2. Methods

We conducted this systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (see Supplementary Table S1 for PRISMA checklist) [13] and the Cochrane Handbook for Systematic Reviews of Interventions [14]. We have registered this systematic review at OSF REGISTRIES (DOI:10.17605/OSF.IO/JC32H, accessed on 3 August 2021).

2.1. Inclusion and Exclusion Criteria

We included published studies and ongoing clinical studies related to the safety, immunogenicity, and efficacy of COVID-19 vaccine in children or adolescents (aged < 18 years). The study design was limited to primary studies, including randomized clinical trials (RCTs), non-randomized trials, and observational studies. We also included ongoing studies registered at the International Clinical Trials Registry Platform (ICTRP).

We excluded articles from which we could not extract data specifically on children or adolescents or if we could not access the full text, conference proceedings, and study protocols. For ongoing studies, we only included registration records if the aim of the study was to determine the safety, immunogenicity, or efficacy of COVID-19 vaccine in children and adolescents.

2.2. Search Strategy

We systematically searched Medline (via PubMed), Web of Science, World Health Organization (WHO) COVID-19 database, and China National Knowledge Infrastructure (CNKI), from their inception to 23 July 2021 to identify studies that met our eligibility criteria. The search strategy combined terms from three themes: (1) COVID-19, (2) vaccine, and (3) children and adolescents (see detailed search strategy in Supplementary Table S2). All search strategies were developed and retrieved independently by two investigators (ML and XL) and then cross-checked. We first developed a search strategy for Medline, and after reaching agreement adapted this strategy for other databases. In addition to the literature databases, we searched ICTRP to identify ongoing studies. We also searched Google Scholar and reference lists of identified articles to avoid missing potentially relevant literature.

2.3. Literature Screening

The screening process included three phases. First, one investigator removed duplicates from the retrieved records. Following this, four investigators (ML, XL, RL, and QS) screened all identified records independently by reading titles and abstracts. If the information in the title and abstract was insufficient, the full text was obtained for review. Disagreements were solved by consensus with the senior researcher (YC). We used Endnote 20.0.1 software in the entire screening process.

2.4. Data Extraction

The following data were extracted from the completed studies: (1) basic information: publication date, country, study design, name of the vaccine; (2) information of the participants: age, sample size, sex distribution; and (3) outcome information: safety, immunogenicity, and efficacy of COVID-19. For the ongoing clinical studies, we extracted the registration date, country, recruitment status, participants' age, target sample size, intervention, and primary outcome. All data were independently extracted by two investigators (ML and XL) using a predesigned extraction sheet.

2.5. Risk of Bias Assessment

Two investigators (ML and XL) assessed the methodological quality of the original studies to ensure the reliability of the findings. We used the Risk of Bias tool recommended by Cochrane Collaboration [15] to assess randomized trials. The tool consists of six domains of bias (selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias). For case-control and cohort studies we used the Newcastle-Ottawa Scale (NOS) [16].; for case series and case reports the checklist proposed by Murad et al. [17]; and for cross-sectional studies the checklist of the Joanna Briggs Institute (JBI) [18].

2.6. Data Analysis

We descriptively presented the main findings on safety, immunogenicity, and efficacy of COVID-19 vaccine in children or adolescents. Microsoft Excel 16.51 (2019) was used for data processing and analysis. We considered to conduct a quantitative meta-analysis if at least two studies were included and the heterogeneity between the studies in terms of outcomes, population characteristics, and type of vaccine was low ($I^2 \leq 50\%$). For ongoing clinical studies, we also presented the numbers of trials by country and type of vaccine. Adobe Illustrator was used to visually present the number of ongoing clinical trials of COVID-19 vaccine in children or adolescents worldwide.

3. Results

3.1. Literature Search

Our initial search revealed 3092 records, of which 931 were excluded as duplicates. After screening the titles and, if necessary, full texts, eight published studies [19–26] with 2852 children or adolescents and 28 ongoing clinical studies targeting to recruit a total of 122,442 participants were included. The study selection process is shown in detail in Figure 1.

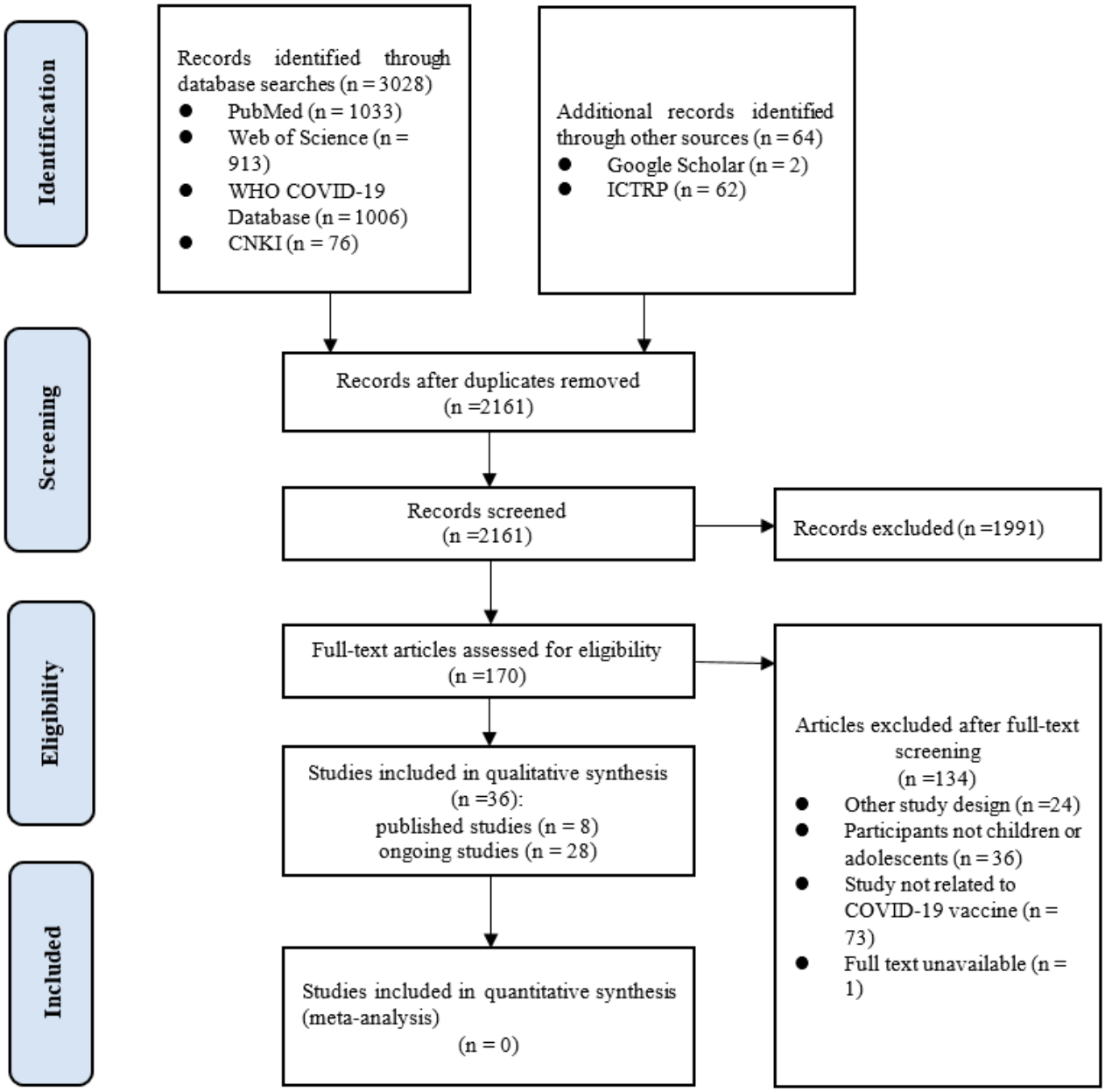


Figure 1. Study selection process (WHO: World Health Organization; COVID-19: coronavirus disease 2019; CNKI: China National Knowledge Infrastructure; ICTRP: International Clinical Trials Registry Platform).

3.2. Characteristics of the Included Clinical Studies

Among the eight published studies included, two were RCTs [19,20], two were case series [21,22], and four were case reports [23–26]. Five studies were conducted in the United States, and one in China, France, and Israel each. The studies were restricted to adolescents with the exception of one RCT that included children aged between 3 and 17 years. In one study the participants received CoronaVac COVID-19 vaccine developed by Sinovac Life Sciences, and in the other seven the participants received BNT162b2 mRNA COVID-19 vaccine developed by Pfizer-BioNTech. The characteristics of the included studies are summarized in Table 1.

Table 1. Basic characteristics of included clinical studies (n = 8).

Name of Vaccine	Participants	Sample Size	Follow-Up Duration	Study Design	Country	Funding	Reference
CoronaVac	Healthy children and adolescents aged 3–17 years	552	4.1 months	RCT Phase 1–2	China	Public/nonprofit (Chinese National Key Research and Development Program and Beijing Science and Technology Program)	Han et al., 2021 [19]
BNT162b2	Adolescents aged 12–15 years with no previous COVID-19 diagnosis or SARS-CoV-2 infection	2264	4.7 months	RCT Phase 3	USA	Private (BioNTech and Pfizer)	Frenck et al., 2021 [20]
BNT162b2	Adolescents and young adults aged 16 years with solid tumor older than	9	NR *	Case series	France	NR *	Riviere et al., 2021 [21]
BNT162b2	Adolescents aged 16–18 years	7	NR *	Case series	Israel	None	Snapiri et al., 2021 [22]
BNT162b2	An adolescent aged 17 years	1	2 weeks	Case report	USA	NR *	Minocha et al., 2021 [23]
BNT162b2	A previously healthy adolescent aged 16 years	1	2 weeks	Case report	USA	NR *	McLean et al., 2021 [24]
BNT162b2	Healthy adolescents 14–18 years	5	unclear	Case report	USA	None	Marshall et al., 2021 [25]
BNT162b2	Children and adolescents aged 12–17 years	13	3 months	Case report	USA	NR *	Schauer et al., 2021 [26]

* NR: not reported.

3.3. Quality of Included Studies

The overall methodological quality of the two included RCTs was high and the risk of bias low (Table 2). In the rest of the studies (case series and case reports), we did not assess two of the eight items of the Murad et al. [17] checklist, "Was there a challenge/rechallenge phenomenon" and "Was there a dose-response effect?", because they were not applicable. One study complied with five of the remaining six items, three with four items, one with three items, and one with two items. The method of case selection was unclear in all

included case series and case reports. Only two case reports or case series reported the item "were other alternative causes that may explain the observation ruled out?", and in three studies the follow-up time was not long enough for outcomes to occur.

Table 2. Quality assessment of included studies.

Risk of Bias in the Included Rcts Assessed by the Risk of Bias Tool							
Selection bias		Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias	Study
Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Anything else, ideally pre-specified	
low	low	low	low	low	low	low	Han et al., 2021 [19]
low	low	low	low	unclear	low	low	Frenck et al., 2021 [20]
Methdological quality in the case series and case reports assessed by Murad et al. checklist							
Selection	Ascertainment		Causality			Reporting	Study
Does the patient(s) represent(s) the whole experience of the investigator (centre) or is the selection method unclear to the extent that other patients with similar presentation may not have been reported?	Was the exposure adequately ascertained?	Was the outcome adequately ascertained?	Were other alternative causes that may explain the observation ruled out?	Was there a challenge/rechallenge phenomenon?	Was there a dose-response effect?	Was follow-up long enough for outcomes to occur?	Is the case(s) described with sufficient details to allow other investigators to replicate the research or to allow practitioners make inferences related to their own practice?	
0	1	1	0	N/A	N/A	0	0	Revon-Riviere et al., 2021 [21]
0	1	1	0	N/A	N/A	0	1	Snapiri et al., 2021 [22]
0	1	1	0	N/A	N/A	1	1	Minocha et al., 2021 [23]
0	1	1	0	N/A	N/A	1	1	McLean et al., 2021 [24]
0	1	1	1	N/A	N/A	0	1	Marshall et al., 2021 [25]
0	1	1	1	N/A	N/A	1	1	Schauer et al., 2021 [26]

0 = no; 1 = yes; N/A: Not applicable.

3.4. Safety of COVID-19 Vaccines

The most common adverse event in the two RCTs was injection site pain [20,21]. Besides that, fever, headache, and fatigue were also frequently reported. Most adverse events were not severe. No deaths were reported. A case series [22] that included 13 patients with solid tumor also showed that mild-to-moderate injection site pain was the most frequent adverse event (6 patients).

Besides, a few diagnosed myocarditis and/or pericarditis cases related to COVID-19 vaccine were reported in some studies. All cases occurred following the second dose of BNT162b mRNA COVID-19 vaccination. We summarized the basic information of 27 cases from included studies (Table 3). The median age was 16 years (range, 12–17 years). Most patients were male (26, 96.3%). Median time of onset was 3 days after receiving the vaccine (range, 1–4 days). All patients had chest pain.

Table 3. Basic information of diagnosed myocarditis and/or pericarditis cases (n = 27).

Vaccination	Age	Sex	Symptoms	Diagnosis	Time of Onset (Days Since Vaccination)	Length of Hospitalization (Days)	Study
BNT162b2, second dose	17	M	Chest pain	Perimyocarditis	3	4	Snapiri et al., 2021 [22]
BNT162b2, second dose	16	M	Chest pain	Perimyocarditis	1	6	Snapiri et al., 2021 [22]
BNT162b2, second dose	16	M	Chest pain, cough	Perimyocarditis	2	6	Snapiri et al., 2021 [22]
BNT162b2, second dose	16	M	Chest pain, nausea	Perimyocarditis	3	4	Snapiri et al., 2021 [22]
BNT162b2, second dose	17	M	Chest pain, headache	Perimyocarditis	1	5	Snapiri et al., 2021 [22]
BNT162b2, second dose	16	M	Chest pain, dyspnea, diarrhea, fever	Perimyocarditis	2	5	Snapiri et al., 2021 [22]
BNT162b2, second dose	17	M	Chest pain, dyspnea	Perimyocarditis	3	3	Snapiri et al., 2021 [22]
BNT162b2, second dose	17	M	Chest pain, fever, body aches,	Myocarditis	1	6	Minocha et al., 2021 [23]
BNT162b2, second dose	16	M	Chest pain	Myopericarditis	2.5	6	McLean et al., 2021 [24]
BNT162b2, second dose	16	M	Chest pain, bilateral arm pain, fever, fatigue, nausea, vomiting, anorexia, headache	Myocarditis	2	6	Marshall et al., 2021 [25]
BNT162b2, second dose	17	M	Chest pain, bilateral arm pain, numbness, paresthesia	Myopericarditis	2	2	Marshall et al., 2021 [25]
BNT162b2, second dose	17	M	Chest pain, bilateral arm pain, abdominal pain, fever, nausea, vomiting, anorexia, SOB, palpitations	Myocarditis	4	5	Marshall et al., 2021 [25]
BNT162b2, second dose	16	M	Chest pain, SOB	Myocarditis	3	3	Marshall et al., 2021 [25]
BNT162b2, second dose	14	M	Chest pain, fever, SOB	Myopericarditis	2	4	Marshall et al., 2021 [25]
BNT162b2, second dose	16	M	Chest pain, fever, chills, myalgias, headache, SOB	Myopericarditis	2	1	Schauer et al., 2021 [26]
BNT162b2, second dose	16	M	Chest pain, fever, myalgias	Myopericarditis	2	1	Schauer et al., 2021 [26]
BNT162b2, second dose	16	M	Chest pain, myalgias, headache	Myopericarditis	3	3	Schauer et al., 2021 [26]
BNT162b2, second dose	17	M	Chest pain, fever, malaise	Myopericarditis	3	1	Schauer et al., 2021 [26]
BNT162b2, second dose	15	M	Chest pain, myalgias, SOB	Myopericarditis	2	2	Schauer et al., 2021 [26]
BNT162b2, second dose	15	F	Chest pain, vomiting	Myopericarditis	3	1	Schauer et al., 2021 [26]
BNT162b2, second dose	15	M	Chest pain, fevers, SOB	Myopericarditis	3	3	Schauer et al., 2021 [26]
BNT162b2, second dose	15	M	Chest pain, chills	Myopericarditis	3	3	Schauer et al., 2021 [26]
BNT162b2, second dose	12	M	Chest pain	Myopericarditis	3	2	Schauer et al., 2021 [26]
BNT162b2, second dose	14	M	Chest pain, fever, headache	Myopericarditis	3	3	Schauer et al., 2021 [26]
BNT162b2, second dose	14	M	Chest pain, malaise, SOB	Myopericarditis	4	2	Schauer et al., 2021 [26]
BNT162b2, second dose	16	M	Chest pain, SOB	Myopericarditis	2	2	Schauer et al., 2021 [26]
BNT162b2, second dose	15	M	Chest pain	Myopericarditis	3	2	Schauer et al., 2021 [26]

M: male; F: female; SOB: shortness of breath.

3.5. Immunogenicity of the COVID-19 Vaccines

The two included RCTs indicated that the investigated COVID-19 vaccines, CoronaVac and BNT162b2, were immunogenic in children and adolescents. Frenck et al. [20] reported that the immune response to BNT162b2 in 12–15 year old adolescents was noninferior to that in young adults aged 16–25 (geometric mean ratio (GMR) = 1.75, 95% CI: 1.47~2.10), indicating even a better response in 12–15 years group than in young adults. Han et al. [19] found that in Phase 1, the seroconversion of neutralizing antibody after the second dose was 100% both in 1.5 μg group and 3.0 μg group with geometric mean titer (GMT) of 55.0 (95% CI 38.9–77.9) and 117.4 (87.8–157.0), respectively (p = 0.0012). In Phase 2, the seroconversion rates were 96·8% (95% CI: 93.1–98.8) and 100% (95% CI: 98.0–100.0) in the 1.5 μg group and the 3.0 μg group, respectively (p = 0.030).

3.6. Efficacy of the COVID-19 Vaccines

The RCTs on BNY162b2 [20] showed that the efficacy of the vaccine in children and adolescents was 100% (95% CI: 75.3~100). The other RCT on CoronaVac did not assess vaccine efficacy.

3.7. Ongoing Clinical Studies

We identified 28 ongoing clinical studies with a total target sample size of 122,442 (see Supplementary Table S3 for ongoing clinical trials on COVID-19 vaccination in children and adolescents). Twenty-three were interventional studies (including one Phase 1 trial; six Phase1/2 trials; six Phase 2 trials; four Phase 2/3 trials; three Phase 3 trials; one Phase 4 trial; and one where the phase was not clear) and five were observational studies. The minimum age of eligible participants was 6 months. Twenty-seven studies reported the name of vaccine they planned to use and there were a total of 15 different vaccine candidates of the following five major types: mRNA (13 studies), inactivated (7 studies), protein subunit (four studies), non-replicating viral vector (four studies), and replicating viral vector (one studies).

The interventional clinical trials were being conducted in 15 countries, the highest numbers of planned trials being in China (10 trials, 43.5%) and the United States (9 trials, 39.1%). BNT162b2 was the most common vaccine (6 trials, 26.1%). Figure 2 shows the countries with ongoing clinical trials and vaccines used in trials.

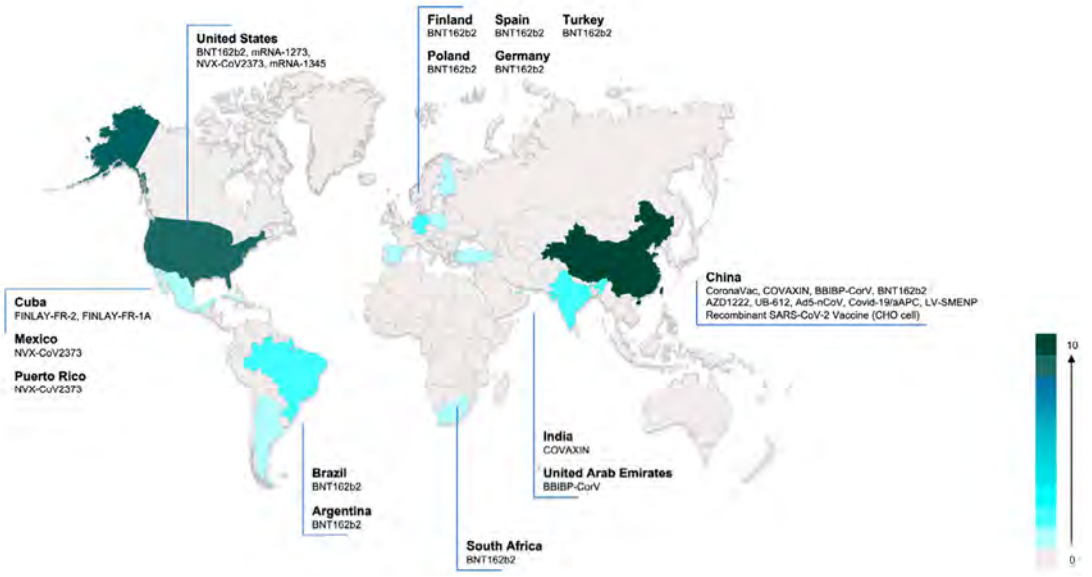


Figure 2. Ongoing interventional COVID-19 vaccine trials in children and adolescents worldwide. Color in the figure indicates the number of ongoing vaccine trials in each country.

4. Discussion

4.1. Principal Findings

Our review identified eight completed studies and 28 ongoing clinical studies of COVID-19 vaccines in children and adolescents. The investigated COVID-19 vaccines had good safety profiles, most adverse effects were mild or moderate, such as injection site pain, fatigue, headache, and chest pain. Some studies reported a few cases of myocarditis and pericarditis. The immune response to the BNT162b2 vaccine in adolescents aged 12–15 years was non-inferior to that in young people aged 16–25 years, and CoronaVac injection had a stronger immune response with a 3.0 μg than 1.5 μg dose. According to the one RCT on BNT162b2, no cases of COVID-19 in adolescents aged 12–15 years were detected. Clinical trials on children and adolescents are being conducted all over the world with a large number of different vaccines.

Children and adolescents, as a special population, present many influencing factors to consider when administering vaccines. Vaccine efficacy and safety are the most important considerations for children and their parents [27]. It is therefore important to demonstrate that vaccines are safe and protective before they are administered to children and adolescents. During an average influenza season, approximately 9.8% of children aged 0–14 year present with influenza [28]. After vaccination against influenza A (H1N1), 90.3% of children and adolescents aged 10–17 years developed protective antibodies, and no serious adverse reactions were seen [29,30]. Similarly, when the COVID-19 outbreak emerged, researchers actively promoted the development of vaccines with the expectation that vaccination could protect healthy population. Our study showed that two vaccines have shown to be effective and safe in pediatric populations. However, the evidence for both vaccines was based on single RCTs, and these two studies both had limitations such as the small sample size and lack of long-term data on safety and immunogenicity data. In particular, the risk of myocarditis and pericarditis should be closely monitored. Most cases of myocarditis and pericarditis associated with the COVID-19 vaccine were mild, and mostly affected children were male. Schauer et al. [26] estimated an incidence of myopericarditis of 0.008% in adolescents 16–17 years of age and 0.01% in those aged 12 through 15 years following the second dose.

Another important factor to consider for vaccination of children and adolescents is the risk of multisystemic inflammatory syndrome in children (MIS-C). In April 2020, children infected with SARS-CoV-2 presenting symptoms similar to incomplete Kawasaki disease (KD) or toxic shock syndrome were documented in the UK [31]. Since then, children with similar symptoms have been reported in other parts of the world as well [32–34]. This condition was subsequently named as MIS-C. The overall mortality of MIS-C is approximately 1–2% [35]. The decision to vaccinate should be made by weighing the risk of exposure, reinfection, and severe disease following infection against the uncertain safety of vaccination in such individuals. Whereas no directly relevant studies have confirmed the association of MIS-C with COVID-19 vaccination, a systematic review published in 2017 [36] identified 27 observational studies and case reports of KD. These showed that diphtheria-tetanus-pertussis (DTP)-containing vaccines, Haemophilus influenzae type b (Hib) conjugate vaccine, influenza vaccine, hepatitis B vaccine, 4-component meningococcal serogroup B (4CMenB) vaccine, measles-mumps-rubella (MMR)/MMR-varicella vaccines, pneumococcal conjugate vaccine (PCV), rotavirus vaccine (RV), yellow fever vaccine, and Japanese encephalitis vaccine did not increase the risk of KD. Thus, children and adolescents at high risk of severe COVID-19 or those with specific comorbidities should be considered to be prioritized in vaccination. More research is needed to clarify to what extent COVID-19 vaccines can mitigate the risks and bring benefits.

To date, 22 COVID-19 vaccines have been approved throughout the world, more than 1/3 of which are inactivated, and 138 vaccines are under development and exploitation. More than 300 clinical trials of COVID-19 vaccines have been registered or published [37,38]. Studies have shown that most COVID-19 vaccines are safe and effective in adults aged $\geq$ 18 years. Overall, in phase 2 and 3 RCTs, mRNA- and adenoviral vector-based COVID-19

vaccines had 94.6% (95% CI 0.936–0.954) and 80.2% (95% CI 0.56–0.93) efficacy, respectively [3–5], with good acceptability [6] and safety [39]. Only two RCTs on children and adolescents have been published in peer-reviewed journals so far, both of which found that the respective vaccines, BNT162b2 and CoronaVac, are safe and effective. Institutions including WHO, Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the Canadian Pediatric Society have already authorized emergency use of BNT162b2 in children and adolescents aged 12 years and above [40–43]. European Medicines Agency (EMA) has also approved the Spikevax (previously COVID-19 Vaccine Moderna) vaccine for adolescents aged 12 to 17 years, based on the evidence from an ongoing study [44]. Although these guidelines gave recommendations on vaccinating children or adolescents from the perspective of Western countries, we still need to wait for more evidence from more countries and regions to better understand how COVID-19 vaccines work in different populations. With the more than twenty ongoing clinical trials, their findings may continue to offer clues of better protecting younger generations from COVID-19.

Public health authorities in countries that have approved COVID-19 vaccine in children and adolescents should also consider multiple aspects in their decision-making. European Centre for Disease Prevention and Control issued a set of eight interim considerations from the view of the overall potential public health impact of COVID-19 vaccination of adolescents [45]. Opel et al. suggested nine criteria to consider when evaluating antigens for inclusion in mandatory school immunization programs, which were categorized into vaccine-related, disease-related, and implementation-related [11]. We currently know however too little about the performance of COVID-19 vaccines or the epidemiology of SARS-CoV-2 in children to make any definitive judgment about whether COVID-19 vaccine should be mandatory in children, especially those under 12. Authorities should closely monitor and continually assess the benefits and potential risks of vaccination in children and adolescents. In addition, the acceptability of the COVID-19 vaccine among both the children themselves as well as their parents and guardians is a major influencing factor on the likelihood of children getting vaccinated. Studies have shown that approximately 80% of parents were reluctant to enroll their children in clinical studies of the COVID-19 vaccine [46] and approximately half of Chinese parents showed hesitancy on taking the COVID-19 vaccine for their children [47]. Therefore, it is necessary to educate parents and children about the vaccine to increase vaccination rates while ensuring the efficacy and safety of vaccines [48]. Furthermore, factors such as national policy, religion, culture, and other routine immunization procedures need to be taken into account in the administration of COVID-19 vaccine to children.

4.2. Potential Impact for Future Research and Practice

Our study included only two RCTs on COVID-19 vaccination in children and adolescents, one investigating CoronaVac developed by Sinovac and one BNT162b2 developed by Pfizer/BioNTech. For the vast majority of vaccines clinical studies are either ongoing but not completed, or not yet planned. For future research, we recommend paying attention to the following three aspects. First, more clinical studies on the protective efficacy and safety of COVID-19 vaccine in children and adolescents need to be conducted. Second, systematic reviews of factors affecting COVID-19 vaccination in children and adolescents, willingness to be vaccinated, and methods to promote vaccination, are needed. This includes also updating this systematic review when more studies, in particular RCTS, on COVID-19 in children and adolescents become available. Third, evidence-based guidelines for COVID-19 vaccination in children and adolescents are needed to promote and standardize vaccination in children and adolescents. Policymakers should develop policies for COVID-19 vaccination in children and adolescents based on the best current evidence in the future, and parents and guardians should be guided by policies that actively encourage and support their children to be vaccinated against COVID-19.

4.3. Strengths and Limitations

This paper is, to the best of our knowledge, the first systematic review on the safety, immunogenicity, and protective efficacy of COVID-19 vaccination in children and adolescents. We systematically searched key databases and websites to conduct a comprehensive evaluation and analysis of published studies and registry data records. However, this paper also has some limitations. First, we did not conduct a meta-analysis in this study, because of the heterogeneity in participant characteristics, outcomes, and study designs. Second, this study only included articles published in English. However, as the amount of evidence published so far is known to be limited, it is reasonable to expect that the studies we included covered most of the knowledge up to now. Finally, some studies that included children and adolescents did not report the age and outcome among these age groups separately. Given the limited time, we excluded these studies instead of contacting authors to request access to original data.

5. Conclusions

Our review found high rates of immunogenicity and vaccine efficacy in children and adolescents. This is a clear indicators that the vaccines are effective, and the RCTs also did not find any major issues with safety. Nevertheless, awareness is needed to monitor the possible adverse effects. Although most adverse events observed in the trials were mild, we identified a limited number of cases of myocarditis and pericarditis among the vaccinated children and adolescents, from several different studies. This shows also that particularly in the current situation where RCTs are still limited, it is important to include all existing evidence, also from individual case reports, in systematic reviews. Real-world data can also reveal findings that may not be observed in the well-controlled RCT settings. It is crucial that more clinical studies with sufficiently long follow-up time, large sample size, and using different types of vaccine are conducted in the future. Evidence-based guidelines are urgently needed to inform policymakers, children and adolescents, and their parents and guardians about the benefits and risks of vaccination against COVID-19.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/vaccines9101102/s1, Table S1: PRISMA checklist, Table S2: detailed search strategy, Table S3: ongoing clinical trials on COVID-19 vaccination in children and adolescents.

Author Contributions: Conceptualization: Y.C., E.L. and Q.L.; study design: Y.C. and Q.L.; literature search: M.L. and X.L. (Xufei Luo); figures and table: M.L. and X.L. (Xiao Liu); data collection: R.L., Q.S., X.L. (Xufei Luo) and M.L.; data analysis: M.L. and X.L. (Xufei Luo); data interpretation: Y.C., Q.L., E.L. and M.L.; writing: M.L., X.L. (Xufei Luo) and Y.C.; supervision: Y.C., E.L. and Q.L. All authors provided input regarding the direction of the study and the content of the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used during the current study are available from the corresponding author on reasonable request.

Acknowledgments: We appreciate Janne Estill for providing comments and language editing for this review.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. CDC. Vaccines: The Basics. Available online: https://www.cdc.gov/vaccines/vpd/vpd-vac-basics.html (accessed on 8 August 2021).
2. The World Bank. Population Ages 0–14 (% of Total Population). Available online: https://data.worldbank.org/indicator/SP.POP.0014.TO.ZS (accessed on 8 August 2021).

3. Xing, K.; Tu, X.Y.; Liu, M.; Liang, Z.W.; Chen, J.N.; Li, J.J.; Jiang, L.G.; Xing, F.Q.; Jiang, Y. Efficacy and safety of COVID-19 vaccines: A systematic review. *Zhongguo Dang Dai Er Ke Za Zhi* **2021**, *23*, 221–228. [CrossRef] [PubMed]
4. Pormohammad, A.; Zarei, M.; Ghorbani, S.; Mohammadi, M.; Razizadeh, M.H.; Turner, D.L.; Turner, R.J. Efficacy and Safety of COVID-19 Vaccines: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. *Vaccines* **2021**, *9*, 467. [CrossRef] [PubMed]
5. McDonald, I.; Murray, S.M.; Reynolds, C.J.; Altmann, D.M.; Boyton, R.J. Comparative systematic review and meta-analysis of reactogenicity, immunogenicity and efficacy of vaccines against SARS-CoV-2. *NPJ Vaccines* **2021**, *6*, 74. [CrossRef] [PubMed]
6. Wang, Q.; Yang, L.; Jin, H.; Lin, L. Vaccination against COVID-19: A systematic review and meta-analysis of acceptability and its predictors [published online ahead of print, 22 June 2021]. *Prev. Med.* **2021**, *150*, 106694. [CrossRef]
7. Pfaar, O.; Klimek, L.; Hamelmann, E.; Kleine-Tebbe, J.; Taube, C.; Wagenmann, M.; Werfel, T.; Brehler, R.; Novak, N.; Mülleneisen, N.; et al. COVID-19 vaccination of patients with allergies and type-2 inflammation with concurrent antibody therapy (biologicals)–A Position Paper of the German Society of Allergology and Clinical Immunology (DGAKI) and the German Society for Applied Allergology (AeDA). *Allergol. Select.* **2021**, *5*, 140–147. [CrossRef]
8. Hazlewood, G.S.; Pardo, J.P.; Barnabe, C.; Schieir, O.; Barber, C.; Bernatsky, S.; Colmegna, I.; Hitchon, C.; Loeb, M.; Mertz, D.; et al. Canadian Rheumatology Association Recommendation for the Use of COVID-19 Vaccination for Patients with Autoimmune Rheumatic Diseases. *J. Rheumatol.* **2021**, *48*, 1330–1339. [CrossRef]
9. WHO. Interim Recommendations for Use of the Inactivated COVID-19 Vaccine, CoronaVac, Developed by Sinovac. 2021. Available online: https://www.who.int/publications/i/item/WHO-2019-nCoV-vaccines-SAGE_recommendation-Sinovac-CoronaVac-2021.1 (accessed on 8 August 2021).
10. Zimmermann, P.; Curtis, N. Why is COVID-19 less severe in children? A review of the proposed mechanisms underlying the age-related difference in severity of SARS-CoV-2 infections. *Arch. Dis. Child.* **2021**, *106*, 429–439. [CrossRef]
11. Opel, D.J.; Diekema, D.S.; Ross, L.F. Should We Mandate a COVID-19 Vaccine for Children? *JAMA Pediatrics* **2021**, *175*, 125–126. [CrossRef]
12. Zhou, Q.; Li, W.; Zhao, S.; Li, Q.; Shi, Q.; Wang, Z.; Liu, H.; Liu, X.; Estill, J.; Luo, Z.; et al. Guidelines for the Management of Children and Adolescent with COVID-19: Protocol for an update. *Transl. Pediatrics* **2021**, *10*, 177–182. [CrossRef]
13. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G.; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *BMJ* **2009**, *339*, b2535. [CrossRef]
14. Higgins, J.; Thomas, J. Cochrane Handbook for Systematic Reviews of Interventions. 2021. Available online: https://training.cochrane.org/handbook/current (accessed on 8 August 2021).
15. Higgins, J.P.T.; Altman, D.G.; Gøtzsche, P.C.; Jüni, P.; Moher, D.; Oxman, A.D.; Savovic, J.; Schulz, K.F.; Weeks, L.; Sterne, J.A.; et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* **2011**, *343*, d5928. [CrossRef]
16. Wells, G.; Shea, B.; O'Connell, D.; Robertson, J.; Peterson, J.; Welch, V.; Losos, M.; Tugwell, P. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses*; Ottawa Hospital Research Institute: Ottawa, ON, Canada, 2011; pp. 1–12.
17. Murad, M.H.; Sultan, S.; Haffar, S.; Bazerbachi, F. Methodological quality and synthesis of case series and case reports. *BMJ Evid.-Based Med.* **2018**, *23*, 60–63. [CrossRef]
18. Joanna Briggs Institute. *Checklist for Analytical Cross Sectional Studies*; The Joanna Briggs Institute: Adelaide, Australia, 2017.
19. Han, B.; Song, Y.; Li, C.; Yang, W.; Ma, Q.; Jiang, Z.; Li, M.; Lian, X.; Jiao, W.; Wang, L.; et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: A double-blind, randomised, controlled, phase 1/2 clinical trial. *Lancet Infect. Dis.* **2021**, *21*, 181–192. [CrossRef]
20. Frenck, R.W., Jr.; Klein, N.P.; Kitchin, N.; Gurtman, A.; Absalon, J.; Lockhart, S.; Perez, J.L.; Walter, E.B.; Senders, S.; Bailey, R.; et al. Safety, immunogenicity, and efficacy of the BNT162b2 COVID-19 vaccine in adolescents. *N. Engl. J. Med.* **2021**, *385*, 239–250. [CrossRef]
21. Revon-Riviere, G.; Ninove, L.; Min, V.; Rome, A.; Coze, C.; Verschuur, A.; de Lamballerie, X.; André, N. BNT162b2 mRNA COVID-19 Vaccine in AYA with cancer: A monocentric experience. *Eur. J. Cancer* **2021**, *154*, 30–34. [CrossRef]
22. Snapiri, O.; Shirman, N.; Weissbach, A.; Weissbach, A.; Lowenthal, A.; Ayalon, I.; Adam, D.; Yarden-Bilavsky, H.; Bilavsky, E. Transient Cardiac Injury in Adolescents Receiving the BNT162b2 mRNA COVID-19 Vaccine. *Pediatric Infect. Dis. J.* **2021**, *40*, e360–e363. [CrossRef]
23. Minocha, P.K.; Better, D.; Singh, R.K.; Hoque, T. Recurrence of Acute Myocarditis Temporally Associated With Receipt of the mRNA COVID-19 Vaccine in an Adolescent Male. *J. Pediatrics* **2021**. [CrossRef]
24. McLean, K.; Johnson, T.J. Myopericarditis in a Previously Healthy Adolescent Male Following COVID-19 Vaccination: A Case Report. *Acad. Emerg. Med.* **2021**. [CrossRef] [PubMed]
25. Marshall, M.; Ferguson, I.D.; Lewis, P.; Jaggi, P.; Gagliardo, C.; Collins, J.S.; Shaughnessy, R.; Caron, R.; Fuss, C.; Corbin, K.; et al. Symptomatic acute myocarditis in seven adolescents following Pfizer-BioNTech COVID-19 vaccination. *Pediatrics* **2021**. [CrossRef] [PubMed]
26. Schauer, J.; Buddhe, S.; Colyer, J.; Sagiv, E.; Law, Y.; Chikkabyrappa, S.M.; Portman, M.A. Myopericarditis after the Pfizer mRNA COVID-19 Vaccine in Adolescents. *J. Pediatrics* **2021**. [CrossRef] [PubMed]
27. Smith, L.E.; Amlôt, R.; Weinman, J.; Yiend, J.; Rubin, G.J. A systematic review of factors affecting vaccine uptake in young children. *Vaccine* **2017**, *35*, 6059–6069. [CrossRef] [PubMed]

28. Ruf, B.R.; Knuf, M. The burden of seasonal and pandemic influenza in infants and children. *Eur. J. Pediatr.* **2014**, *173*, 265–276. [CrossRef] [PubMed]
29. Wijnans, L.; de Bie, S.; Dieleman, J.; Bonhoeffer, J.; Sturkenboom, M. Safety of pandemic H1N1 vaccines in children and adolescents. *Vaccine* **2011**, *29*, 7559–7571. [CrossRef]
30. Lu, C.Y.; Shao, P.L.; Chang, L.Y.; Huang, Y.C.; Chiu, C.H.; Hsieh, Y.C.; Lin, T.Y.; Huang, L.M. Immunogenicity and safety of a monovalent vaccine for the 2009 pandemic influenza virus A (H1N1) in children and adolescents. *Vaccine* **2010**, *28*, 5864–5870. [CrossRef] [PubMed]
31. Riphagen, S.; Gomez, X.; Gonzalez-Martinez, C.; Wilkinson, N.; Theocharis, P. Hyperinflammatory shock in children during COVID-19 pandemic. *Lancet* **2020**, *395*, 1607. [CrossRef]
32. Licciardi, F.; Pruccoli, G.; Denina, M.; Parodi, E.; Taglietto, M.; Rosati, S.; Montin, D. SARS-CoV-2-Induced Kawasaki-Like Hyperinflammatory Syndrome: A Novel COVID Phenotype in Children. *Pediatrics* **2020**, *146*, e20201711. [CrossRef] [PubMed]
33. Verdoni, L.; Mazza, A.; Gervasoni, A.; Martelli, L.; Ruggeri, M.; Ciuffreda, M.; Bonanomi, E.; D'Antiga, L. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: An observational cohort study. *Lancet* **2020**, *395*, 1771. [CrossRef]
34. Whittaker, E.; Bamford, A.; Kenny, J.; Kaforou, M.; Jones, C.E.; Shah, P.; Ramnarayan, P.; Fraisse, A.; Miller, O.; Davies, P.; et al. Clinical Characteristics of 58 Children with a Pediatric Inflammatory Multisystem Syndrome Temporally Associated With SARS-CoV-2. *JAMA* **2020**, *324*, 259. [CrossRef]
35. Kaushik, A.; Gupta, S.; Sood, M.; Sharma, S.; Verma, S. A Systematic Review of Multisystem Inflammatory Syndrome in Children Associated With SARS-CoV-2 Infection. *Pediatr. Infect. Dis. J.* **2020**, *39*, e340–e346. [CrossRef]
36. Phuong, L.K. Kawasaki disease and immunisation: A systematic review. *Vaccine* **2017**, *35*, 1770–1779. [CrossRef]
37. Vaccines Candidates by Trial Phase. Available online: https://covid19.trackvaccines.org/vaccines (accessed on 8 August 2021).
38. COVID-19 NMA. Available online: https://covid-nma.com/vaccines/mapping/ (accessed on 8 August 2021).
39. Kaur, R.J.; Dutta, S.; Bhardwaj, P.; Charan, J.; Dhingra, S.; Mitra, P.; Singh, K.; Yadav, D.; Sharma, P.; Misra, S. Adverse Events Reported From COVID-19 Vaccine Trials: A Systematic Review [published online ahead of print, 2021 Mar 27]. *Indian J. Clin. Biochem.* **2021**, 1–13. [CrossRef]
40. World Health Organization. *Interim Recommendations for Use of the Pfizer–BioNTech COVID-19 Vaccine, BNT162b2, under Emergency Use Listing: Interim Guidance, First Issued 8 January 2021, Updated 15 June 2021[R]*; World Health Organization: Geneva, Switzerland, 2021.
41. Centers for Disease Control and Prevention. COVID-19 Vaccines for Children and Teens. Updated 23 July 2021. Available online: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/recommendations/adolescents.html (accessed on 8 August 2021).
42. Food and Drug Administration. *Pfizer-BioNTech COVID-19 Vaccine Emergency Use Authorization*; US Department of Health and Human Services, Food and Drug Administration: Silver Spring, MD, USA, 2021. Available online: https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/pfizer-biontech-covid-19-vaccine (accessed on 8 August 2021).
43. The Canadian Paediatric Society. COVID-19 Vaccine for Children. Updated 12 July 2021. Available online: https://www.cps.ca/en/documents/position/covid-19-vaccine-for-children (accessed on 8 August 2021).
44. European Medicines Agency. COVID-19 Vaccine Spikevax Approved for Children Aged 12 to 17 in EU. 23 July 2021. Available online: https://www.ema.europa.eu/en/news/covid-19-vaccine-spikevax-approved-children-aged-12-17-eu (accessed on 8 August 2021).
45. European Center for Disease Prevention and Control. *Interim Public Health Considerations for COVID-19 Vaccination of Adolescents in the EU/EEA*; ECDC: Stockholm, Sweden, 1 June 2021. Available online: https://www.ecdc.europa.eu/en/publications-data/interim-public-health-considerations-covid-19-vaccination-adolescents-eueea#no-link (accessed on 8 August 2021).
46. Goldman, R.D.; Staubli, G.; Cotanda, C.P.; Brown, J.C.; Hoeffe, J.; Seiler, M.; Gelernter, R.; Hall, J.E.; Griffiths, M.A.; Davis, A.L.; et al. Factors Associated with Parents' Willingness to Enroll Their Children in Trials for COVID-19 Vaccination. *Hum. Vaccin. Immunother.* **2021**, *17*, 1607–1611. [CrossRef] [PubMed]
47. Wang, Q.; Xiu, S.; Zhao, S.; Wang, J.; Han, Y.; Dong, S.; Huang, J.; Cui, T.; Yang, L.; Shi, N.; et al. Vaccine Hesitancy: COVID-19 and Influenza Vaccine Willingness among Parents in Wuxi, China-A Cross-Sectional Study. *Vaccines* **2021**, *9*, 342. [CrossRef] [PubMed]
48. Nour, R. A Systematic Review of Methods to Improve Attitudes towards Childhood Vaccinations. *Cureus* **2019**, *11*, e5067. [CrossRef] [PubMed]

6.12. Estudo com mais de dez milhões de chilenos maiores de 16 anos mostra que efetividade da CoronaVac é superior a 86%

A efetividade da CoronaVac entre adolescentes já é um fato comprovado desde setembro de 2021, quando pesquisadores chilenos publicaram o artigo "Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile" no periódico científico The New England Journal of Medicine, um dos mais prestigiosos do mundo. O estudo, realizado entre fevereiro e maio de 2021 com 10,2 milhões de pessoas, investigou a eficácia da vacina no "mundo real" contra casos de Covid-19 e no combate às variantes do SARS-CoV-2 então circulantes no país – gama e alfa, principalmente.

O estudo de coorte (pesquisa observacional que acompanha indivíduos ao longo de um período de tempo para determinar características e evolução do grupo) contou com participantes acima dos 16 anos cadastrados no Fundo Nacional de Saúde (FONASA), programa nacional de saúde chileno que cobre cerca de 80% da população. O esquema vacinal aplicado no país é de duas doses da CoronaVac com intervalo de 28 dias.

A pesquisa mostrou que a proteção da vacina do Butantan e da Sinovac foi de 65,9% contra infecções por Covid-19, de 87,5% contra hospitalizações, de 90,3% contra internações em Unidades de Terapia Intensiva (UTI) e de 86,3% contra mortes.

Participaram do estudo 708.676 jovens de 16 a 19 anos, o equivalente a 7% do total de voluntários do coorte. Destes, 8.192 (1,2%) receberam uma dose de CoronaVac e 30.033 (4,2%) receberam duas doses. Os demais 670.451 consistiam em grupo controle ou pessoas que haviam tido Covid-19 (14.871). Vale ressaltar que, no Chile, assim como no Brasil, a vacinação foi iniciada pelos idosos, considerados mais vulneráveis à Covid-19.

O país andino tem as taxas mais elevadas de realização de testes para detecção da Covid-19 na América Latina e um sistema padronizado de informação pública para estatísticas vitais ao estudo. Na época, o Ministério da Saúde chileno já havia utilizado 13,98 milhões de doses da CoronaVac desde o começo da campanha de vacinação, em fevereiro.

Publicado em: 2/9/2021

Artigo

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 SEPTEMBER 2, 2021 VOL. 385 NO. 10

Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile

Alejandro Jara, Ph.D., Eduardo A. Undurraga, Ph.D., Cecilia González, M.D., Fabio Paredes, M.Sc., Tomás Fontecilla, M.Sc., Gonzalo Jara, B.S.E., Alejandra Pizarro, M.D., Johanna Acevedo, M.S., Katherinne Leo, B.S.E., Francisco Leon, M.B.A., Carlos Sans, B.S.E., Paulina Leighton, B.S.E., Pamela Suárez, B.S.E., Heriberto Garda-Escorza, M.S., and Rafael Araos, M.D.

ABSTRACT

BACKGROUND

Mass vaccination campaigns to prevent coronavirus disease 2019 (Covid-19) are occurring in many countries; estimates of vaccine effectiveness are urgently needed to support decision making. A countrywide mass vaccination campaign with the use of an inactivated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine (CoronaVac) was conducted in Chile starting on February 2, 2021.

METHODS

We used a prospective national cohort, including participants 16 years of age or older who were affiliated with the public national health care system, to assess the effectiveness of the inactivated SARS-CoV-2 vaccine with regard to preventing Covid-19 and related hospitalization, admission to the intensive care unit (ICU), and death. We estimated hazard ratios using the extension of the Cox proportional-hazards model, accounting for time-varying vaccination status. We estimated the change in the hazard ratio associated with partial immunization (≥14 days after receipt of the first dose and before receipt of the second dose) and full immunization (≥14 days after receipt of the second dose). Vaccine effectiveness was estimated with adjustment for individual demographic and clinical characteristics.

RESULTS

The study was conducted from February 2 through May 1, 2021, and the cohort included approximately 10.2 million persons. Among persons who were fully immunized, the adjusted vaccine effectiveness was 65.9% (95% confidence interval [CI], 65.2 to 66.6) for the prevention of Covid-19 and 87.5% (95% CI, 86.7 to 88.2) for the prevention of hospitalization, 90.3% (95% CI, 89.1 to 91.4) for the prevention of ICU admission, and 86.3% (95% CI, 84.5 to 87.9) for the prevention of Covid-19–related death.

CONCLUSIONS

Our results suggest that the inactivated SARS-CoV-2 vaccine effectively prevented Covid-19, including severe disease and death, a finding that is consistent with results of phase 2 trials of the vaccine. (Funded by Agencia Nacional de Investigación y Desarrollo and others.)

From the Ministry of Health (A.J., C.G., F.P., T.F., G.J., A.P., J.A., K.L., P.L., P.S., H.G.-E., R.A.), Facultad de Matemáticas (A.J.) and Escuela de Gobierno (E.A.U.), Pontificia Universidad Católica de Chile, Millennium Nucleus Center for the Discovery of Structures in Complex Data (A.J.), Millennium Initiative for Collaborative Research in Bacterial Resistance (E.A.U., R.A.), the Research Center for Integrated Disaster Risk Management (E.A.U.), Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina, Clínica Alemana Universidad del Desarrollo (R.A.), and the Advanced Center for Chronic Diseases (R.A.) — all in Santiago, Chile; and the CIFAR Azrieli Global Scholars Program, CIFAR, Toronto (E.A.U.). Address reprint requests to Dr. Araos at Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina, Clínica Alemana Universidad del Desarrollo, Av. Las Condes 12461, Las Condes 7580943, Chile, or at rafaelaraos@udd.cl.

Drs. Jara, Undurraga, and Araos contributed equally to this article.

This article was published on July 7, 2021, and updated on July 13, 2021, at NEJM.org.

N Engl J Med 2021;385:875-84.
DOI: 10.1056/NEJMoa2107715

THE CORONAVIRUS DISEASE 2019 (COVID-19) pandemic has imposed an enormous disease burden worldwide, with more than 159 million cases and approximately 3.3 million deaths reported as of May 10, 2021.[1] Covid-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and the severity ranges from mild symptoms to life-threatening disease.[2] Older age and underlying conditions substantially increase the case fatality rate.[3,4] Nonpharmaceutical interventions, such as social distancing, face masks, and contact tracing, have so far been the mainstay of health policy strategies to reduce viral spread and limit demands on health care.[5,6] New Covid-19 vaccines are beginning to change this situation. On December 2, 2020, the first vaccine tested in a large, randomized clinical trial was approved in the United Kingdom,[7,8] although some countries began vaccinations before clinical results were available. Several effective vaccines against Covid-19 have been developed and approved in record time,[8-12] and numerous new vaccines are in the final stages of clinical trials.[13]

Mass vaccination campaigns to prevent Covid-19 are now occurring in many countries.[14] Preliminary results of the effectiveness of other Covid-19 vaccines across different populations have been published, including studies at the national level in Israel[15] and Scotland[16] and studies involving essential frontline workers at specific locations in the United States.[17-19] Estimates of vaccine effectiveness in the prevention of Covid-19 are essential because they reflect real-world challenges, such as logistics, cold chains, vaccination schedules, and follow-up, and also involve more diverse populations than those selected in randomized clinical trials, such as older or immunocompromised persons or those with coexisting conditions. Despite being the standard for assessing vaccine efficacy, phase 3 clinical trials have some limitations, such as restrictive inclusion criteria and implementation under strict experimental conditions that may not resemble a mass vaccination rollout.[20] Thus, large observational studies to estimate the effectiveness of new vaccines in real-world settings are an essential complement to randomized, controlled trials.[21]

Existing vaccine-effectiveness estimates have focused on the BNT162b2 messenger RNA (mRNA) vaccine (Pfizer–BioNTech), the ChAdOx1 nCoV-19 vaccine (Oxford–AstraZeneca), and the mRNA-1273 vaccine (Moderna).[15-19] Several countries are conducting vaccination campaigns with the use of an inactivated SARS-CoV-2 vaccine (CoronaVac) amid a record surge of Covid-19 cases worldwide.[1,13] A total of 22 primarily low- and middle-income countries have approved the CoronaVac vaccine for emergency use. Despite its global importance, limited evidence is available on the efficacy or effectiveness of this vaccine.

Phase 1–2 trials of the CoronaVac vaccine[22] were carried out in China among participants 18 to 59 years of age[23] and in participants 60 years of age or older.[24] The findings suggested that the vaccine was safe and immunogenic in most patients 14 days after receipt of the second dose. Phase 3 clinical trials are taking place in Brazil, Chile, Indonesia, and Turkey (ClinicalTrials.gov numbers, NCT04456595, NCT04651790, NCT04508075, and NCT04582344, respectively). Efficacy results from these trials have not yet been published, but reported efficacy estimates from the manufacturers with regard to mild Covid-19 have varied substantially among the sites: 50.7% (95% confidence interval [CI], 35.6 to 62.2) in Brazil, 65.3% in Indonesia, and 83.5% (95% CI, 65.4 to 92.1) in Turkey.[25-28] In addition, preliminary estimates from an observational study involving vaccinated health care workers (from a preprint server) suggested that at least one dose of the CoronaVac vaccine was 49.6% (95% CI, 11.3 to 71.4) effective against Covid-19 in Manaus, Brazil, a location where the P.1 (or gamma) variant, which is considered to be a variant of concern by the Centers for Disease Control and Prevention,[29] is predominant (occurred in approximately 75% of the test results).[30] No estimates of the effectiveness of the CoronaVac vaccine with regard to preventing Covid-19 in the general population or in persons who have received full vaccination are publicly available.

On February 2, 2021, Chile began a mass vaccination campaign with the CoronaVac vaccine (Section S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).[31] The Public Health Institute of Chile approved the CoronaVac vaccine for emergency use on January 20, 2021; the vaccine is to be administered in a two-dose schedule, with doses separated by 28 days. The vaccination campaign prioritized older adults, beginning at 90 years of age or older; frontline health care workers; and persons with underlying conditions. The government relied on the existing health care infrastructure to roll the vaccines out to the eligible

population where they lived. Vaccination rollout was organized by means of a publicly available national schedule that assigned specific dates to eligible groups. Eligible persons needed to show up at the nearest vaccination site with their identification; they did not need to make an appointment (Figs. S3 and S4). A national immunization registry keeps track of the vaccination schedules. As of May 10, 2021, the Ministry of Health has administered 13.98 million doses of the CoronaVac vaccine (7.62 million first doses and 6.36 million second doses).[32] Vaccine introduction and scale-up of the campaign occurred during a period with the highest incidence rates of Covid-19 since the beginning of the pandemic in Chile.

We used a rich administrative observational data set to provide estimates of the effectiveness of the CoronaVac vaccine in preventing Covid-19 and related hospitalization, admission to the intensive care unit (ICU), and death in the Chilean population. We estimated the effectiveness of the administration of one vaccine dose and of two doses (the complete schedule), with adjustment for relevant demographic and clinical confounders of the association between vaccination and Covid-19 outcomes. We conducted robustness checks to test whether vaccine effectiveness would be affected by differences in health care access between the vaccinated and unvaccinated groups, and we provide vaccine-effectiveness estimates among persons 16 to 59 years of age and among those 60 years of age or older.

METHODS

STUDY POPULATION AND DESIGN

We used a prospective observational cohort at the national level. The study cohort included participants 16 years of age or older who were affiliated with Fondo Nacional de Salud (FONASA), the national public health insurance program, which includes approximately 80% of the Chilean population. A detailed description of the vaccination campaign is provided in the Supplementary Appendix. Eligibility criteria included an age of 16 years or more, affiliation with FONASA, and receipt of at least one dose of the CoronaVac vaccine between February 2 and May 1, 2021, or no receipt of any Covid-19 vaccination. We excluded participants with a probable or confirmed SARS-CoV-2 infection, as assessed by reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay or antigen testing, on or before February 2, 2021, and persons who had received at least one dose of the BNT162b2 vaccine. We did not focus on the effectiveness of the BNT162b2 vaccine because these estimates have been provided elsewhere.[15,17] We focused on the results regarding the CoronaVac vaccine because they are the mainstay of the vaccination strategy in Chile. However, we provide estimates of the effectiveness of the BNT162b2 vaccine in the Supplementary Appendix as a validation of the procedures used here.

All persons 16 years of age or older are eligible to receive the vaccine, according to the national vaccination schedule. We classified participants into three groups: those who were not vaccinated, those who were partially immunized (≥14 days after receipt of the first vaccine dose and before receipt of the second dose), and those who were fully immunized (≥14 days after receipt of the second dose).

The study team was entirely responsible for the design of the study and for the collection and analysis of the data. The authors vouch for the accuracy and completeness of the data. The first, second, and last authors wrote the first draft of the manuscript.

OUTCOMES AND COVARIATES

We estimated vaccine effectiveness using four primary outcomes: laboratory-confirmed Covid-19, hospitalization for Covid-19, admission to the ICU for Covid-19, and Covid-19–related death. For all the outcomes, we considered the time from the beginning of follow-up (February 2, 2021) to the onset of symptoms as the end point. Vaccine-effectiveness estimates regarding Covid-19 cases included the more severe outcomes. All suspected cases of Covid-19 in Chile are notified to health authorities by means of an online platform and are confirmed by laboratory testing. In our study, cases of Covid-19 and related deaths were those in persons with laboratory-confirmed infection, which corresponds to code U07.1 in the *International Classification of Diseases, 10th Revision*.

We controlled for several patient characteristics that could confound the association between vaccination and outcomes, including age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19. These conditions included chronic kidney disease, diabetes, cardiovascular disease, stroke, chronic obstructive pulmonary disease, hematologic dis-

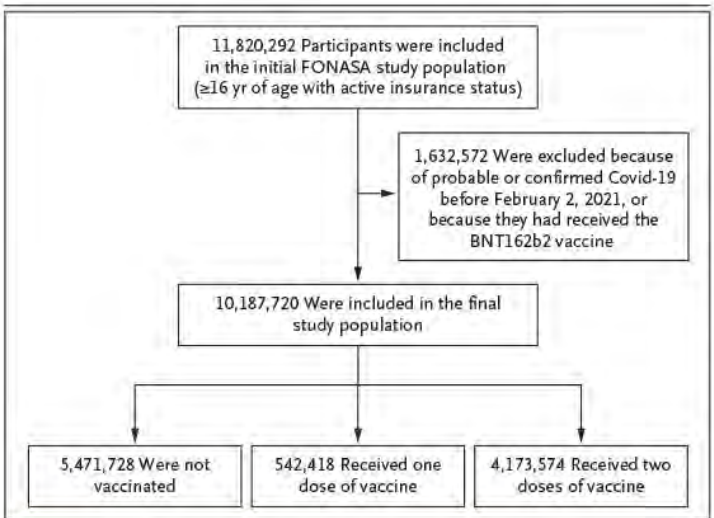


Figure 1. Study Participants and Cohort Eligibility.

Participants were at least 16 years of age, were affiliated with Fondo Nacional de Salud (FONASA; the national public health care system in Chile), and either had received at least one dose of the CoronaVac vaccine between February 2 and May 1, 2021, or had not received any vaccination. We excluded persons who had probable or confirmed coronavirus disease 2019 (Covid-19) according to reverse-transcriptase–polymerase-chain-reaction assay for severe acute respiratory syndrome coronavirus 2 and all persons who had been immunized with the BNT162b2 vaccine.

ease, autoimmune disease, human immunodeficiency virus infection, and Alzheimer's disease and other dementias.[4,33-35]

STATISTICAL ANALYSIS

Our analysis was broadly based on the analytic methods of Thompson et al.[17] for estimating vaccine effectiveness in the United States. We determined vaccine effectiveness by estimating the hazard ratio between the vaccinated and unvaccinated groups. On the basis of the observed information regarding the time to symptom onset from February 2, 2021, we estimated hazard ratios using the extension of the Cox proportional-hazards model, which allowed us to account for a time-varying vaccination status of the persons in the study. We evaluated the robustness of the model assumptions by fitting a stratified version of the extended Cox proportional-hazards model using the available predictors. Inference was based on a partial likelihood approach (Section S2).[17] We estimated the change in the hazard associated with partial immunization and full immunization, and both time-to-event analyses were performed separately. Because the immunity status induced by the CoronaVac vaccine is unknown during the 13 days between vaccine administration and partial or full immunization, those periods were excluded from the at-risk person-time in our analyses.[17]

We estimated the vaccine effectiveness as 1 minus the corresponding hazard ratio, obtained from a model including the previously described covariates, which was expressed as a percentage. We also provide the results with adjustment for the effect of sex and age only. To evaluate whether our effectiveness results were affected by potentially different access to health care between vaccinated persons and unvaccinated persons and according to the age distribution, we performed subgroup analyses involving the subgroup of persons with access to RT-PCR or antigen testing for SARS-CoV-2 and subgroups of persons 60 years of age or older and persons 16 to 59 years of age. Statistical analyses were conducted with the use of the survival package of R software, version 4.0.5.[36,37]

RESULTS

STUDY POPULATION AND VACCINATION ROLLOUT

Figure 1 shows the flow diagram of the study cohort. Of the 11,820,292 persons 16 years of age or older who were affiliated with FONASA, 10,187,720 were eligible for inclusion in the study. Table 1 shows the descriptive statistics for the approximately 10.2 million participants included in the study cohort. There were significant differences according to geographic region, sex, age, income group, nationality, and presence of underlying medical conditions, both in the incidence of Covid-19 and according to vaccination status (unvaccinated, vaccinated with only one dose, or vaccinated with two doses). Laboratory confirmation of infection was by RT-PCR assay in 98.1% of the cases and by antigen testing in 1.9%. Figure 2A shows the rapid rollout of the vaccination campaign, which started on February 2, 2021. Details of the vaccination campaign are provided in Section S1 and Figures S5 through S8. Figure 2B shows the crude cumulative incidence of Covid-19 during the study period among persons who had received one or two doses of vaccine or were unvaccinated.

VACCINE EFFECTIVENESS

There were approximately 615 million person-days in the unvaccinated group, 70 million person-days in the partially immunized group, and 92 million

Table 1. Characteristics of the Study Cohort, Overall and Those with Laboratory-Confirmed Covid-19, According to Vaccination Status.*

Characteristic	Cohort Participants		Persons with Covid-19		P Value	Unvaccinated Persons		Persons Vaccinated with One Dose		Persons Vaccinated with Two Doses		P Value
	no.	%	no.	%		no.	%	no.	%	no.	%	
Total	10,187,720	100	248,645	2.4	—	5,471,728	53.7	542,418	5.3	4,173,574	41.0	—
Sex												<0.001
Female	5,469,202	54.0	135,311	2.5	<0.001	2,775,436	50.8	272,044	5.0	2,421,722	44.3	
Male	4,718,518	46.0	113,334	2.4		2,696,292	57.1	270,374	5.7	1,751,852	37.1	
Age group												<0.001
16–19 yr	708,676	7.0	14,871	2.1	<0.001	670,451	94.6	8,192	1.2	30,033	4.2	
20–29 yr	2,017,676	20.0	59,645	3.0		1,655,595	82.1	55,854	2.8	306,227	15.2	
30–39 yr	1,867,491	18.0	54,480	2.9		1,446,544	77.5	59,166	3.1	361,781	19.4	
40–49 yr	1,423,770	14.0	39,993	2.8		851,622	59.8	165,487	11.6	406,661	28.6	
50–59 yr	1,457,564	14.0	37,539	2.6		434,694	29.8	184,268	12.6	838,602	57.5	
60–69 yr	1,365,940	13.0	23,669	1.7		221,738	16.2	41,693	3.1	1,102,509	80.7	
70–79 yr	870,082	8.5	11,778	1.4		111,592	12.8	16,412	1.9	742,078	85.3	
≥80 yr	476,521	4.7	6,670	1.4		79,492	16.7	11,346	2.4	385,683	80.9	
No. of coexisting conditions†												<0.001
0	6,880,426	68.0	168,401	2.4	0.04	4,447,684	64.6	394,030	5.7	2,038,712	29.6	
≥1	3,307,294	32.0	80,244	2.4		1,024,044	31.0	148,388	4.5	2,134,862	64.6	<0.001
Nationality												
Chilean	9,497,058	93.2	233,572	2.5	<0.001	4,913,208	51.7	513,604	5.4	4,070,246	42.9	
Non-Chilean	690,662	6.8	15,073	2.2		558,520	80.9	28,814	4.2	103,328		

* The study cohort included eligible persons who were affiliated with Fondo Nacional de Salud, the national public health insurance program, which collects, manages, and distributes funds for the public health care system in Chile. The model also included individual-level income and location (16 regions). Additional details are provided in Table S1. Covid-19 denotes coronavirus disease 2019.

† Coexisting conditions included chronic kidney disease, diabetes, cardiovascular disease (hypertension or myocardial infarction), stroke, chronic obstructive pulmonary disease, hematologic disease (lymphoma, leukemia, or myeloma), autoimmune disease (rheumatoid arthritis, juvenile idiopathic arthritis, or systemic lupus erythematosus), human immunodeficiency virus infection, and Alzheimer's disease and other dementias.

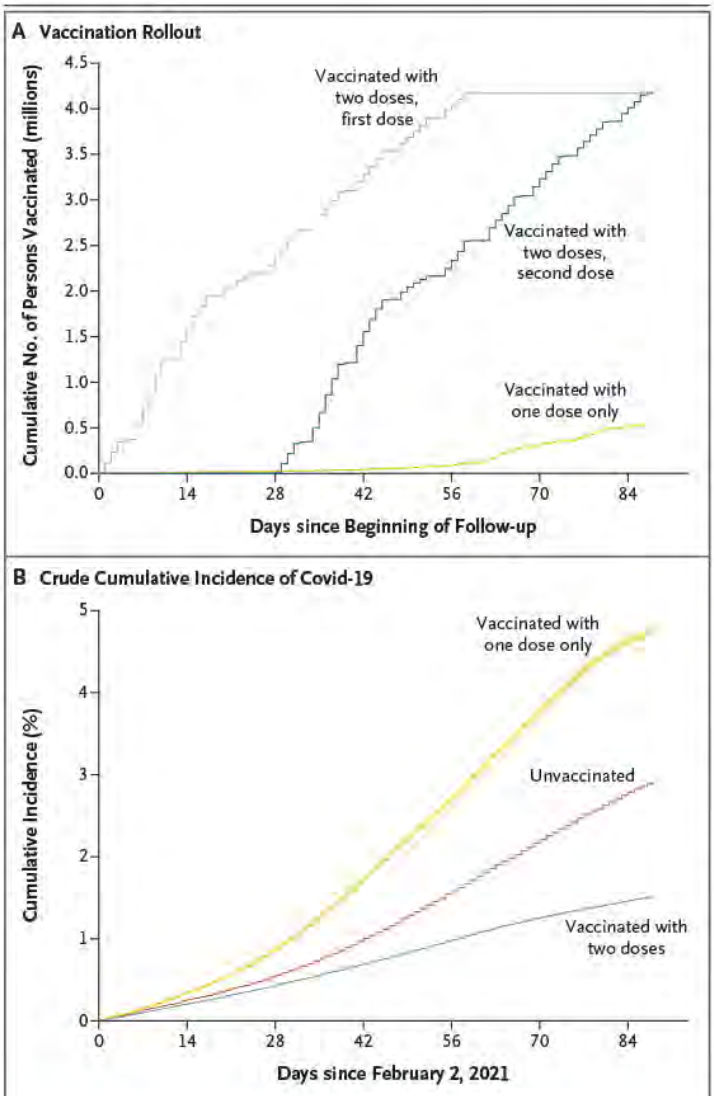


Figure 2. Vaccination Rollout and Crude Cumulative Incidence of Covid-19 in the Study Cohort.

Panel A shows the pace and coverage of the vaccination program among persons who received both doses of vaccine (first and second doses shown separately) or only one dose during the study period (February 2 through May 1, 2021). Panel B shows the crude cumulative incidence of Covid-19 during the study period among unvaccinated persons, among persons who had received only one dose of vaccine, and among persons who had received both doses of vaccine. The relatively high cumulative incidence of Covid-19 in the one-dose group should be interpreted with caution. As shown in Panel A, this group initiated vaccination approximately 40 days after the beginning of the vaccination campaign on February 2, 2021. Therefore, the incidence curve includes all cases that occurred from before vaccination up to 13 days after receipt of the first dose. Shading on the lines indicates 95% confidence intervals.

person-days in the fully immunized group during the study period (Table 2). We documented 218,784 cases of Covid-19, as well as 22,866 hospitalizations, 7873 ICU admissions, and 4042 deaths.

We estimated that the vaccine effectiveness among partially immunized persons (14 to 28 days after receipt of the first dose) was 15.5% (95% CI, 14.2 to 16.8) for the prevention of Covid-19 and 37.4% (95% CI, 34.9 to 39.9) for the prevention of hospitalization, 44.7% (95% CI, 40.8 to 48.3) for the prevention of admission to the ICU, and 45.7% (95% CI, 40.9 to 50.2) for the prevention of Covid-19–related death. In the fully immunized group, the estimated adjusted vaccine effectiveness was 65.9% (95% CI, 65.2 to 66.6) for the prevention of Covid-19 and 87.5% (95% CI, 86.7 to 88.2) for the prevention of hospitalization, 90.3% (95% CI, 89.1 to 91.4) for the prevention of ICU admission, and 86.3% (95% CI, 84.5 to 87.9) for the prevention of Covid-19–related death (Table 2). The vaccine-effectiveness estimates in the stratified model were consistent with these results.

We estimated that the adjusted vaccine effectiveness in the subgroup of fully immunized persons 60 years of age or older was 66.6% (95% CI, 65.4 to 67.8) for the prevention of Covid-19 and 85.3% (95% CI, 84.3 to 86.3) for the prevention of hospitalization, 89.2% (95% CI, 87.6 to 90.6) for the prevention of ICU admission, and 86.5% (95% CI, 84.6 to 88.1) for the prevention of Covid-19–related death (Table 3). Vaccine-effectiveness estimates among persons 16 to 59 years of age are provided in Table S3.

To address a potential concern that the observed vaccine effectiveness may have been driven by health care access, we conducted an analysis in the subgroup of persons who had undergone testing with an RT-PCR assay (98.1%) or antigen test (1.9%) during the analysis period. The results, conditional on whether testing was performed, showed larger effects for vaccination than when we included the complete cohort. Among fully immunized persons in this subgroup, the adjusted vaccine effectiveness was 72.9% (95% CI, 72.3 to 73.4) for the prevention of Covid-19 and 89.2% (95% CI, 88.5 to 89.8) for the prevention of hospitalization, 91.6% (95% CI, 90.5 to 92.5) for the prevention of ICU admission, and 87.8% (95% CI, 86.2 to 89.2) for the prevention of Covid-19–related death (Table S4).

DISCUSSION

We provide estimates of the effectiveness of administration of the CoronaVac vaccine in a countrywide mass vaccination campaign for the prevention of laboratory-confirmed Covid-19 and related hospitalization, admission to the ICU, and

Table 2. Effectiveness of CoronaVac Vaccine in Preventing Covid-19 Outcomes in Overall Study Cohort, According to Immunization Status.*

Outcome and Immunization Status	Study Cohort	Persons with Covid-19		Vaccine Effectiveness (95% CI)		
	No. of Person-Days	No. of Persons	Incidence Rate	Analysis Adjusted for Sex and Age	Analysis Adjusted for All Covariates†	Stratified Analysis‡
			no. of events/ 1000 person-days		*percent*	
Covid-19						
Unvaccinated	614,868,240	185,633	0.3019	—	—	—
Partially immunized	69,788,352	20,865	0.2990	8.0 (6.5–9.4)	15.5 (14.2–16.8)	17.2 (15.8–18.6)
Fully immunized	91,671,797	12,286	0.1340	61.2 (60.3–62.0)	65.9 (65.2–66.6)	63.7 (62.8–64.6)
Hospitalization						
Unvaccinated	620,894,706	18,034	0.0290	—	—	—
Partially immunized	70,690,796	3,370	0.0477	31.4 (28.6–34.0)	37.4 (34.9–39.9)	40.3 (37.6–42.8)
Fully immunized	92,445,333	1,462	0.0158	86.0 (85.1–86.8)	87.5 (86.7–88.2)	86.5 (85.6–87.4)
Admission to ICU						
Unvaccinated	621,226,431	6,359	0.0102	—	—	—
Partially immunized	70,836,597	1,154	0.0163	37.5 (33.1–41.5)	44.7 (40.8–48.3)	45.3 (41.2–49.2)
Fully immunized	92,622,083	360	0.0039	88.8 (87.4–90.0)	90.3 (89.1–91.4)	90.2 (88.9–91.4)
Confirmed death						
Unvaccinated	621,426,477	2,786	0.0045	—	—	—
Partially immunized	70,854,187	847	0.0120	39.8 (34.4–44.7)	45.7 (40.9–50.2)	46.0 (40.7–50.8)
Fully immunized	92,514,261	409	0.0044	84.4 (82.4–86.2)	86.3 (84.5–87.8)	86.7 (84.9–88.3)

* Participants were classified into three groups: those who were unvaccinated, those who were partially immunized (≥14 days after receipt of the first vaccine dose and before receipt of the second dose), and those who were fully immunized (≥14 days after receipt of the second dose). The 13 days between vaccine administration and partial or full immunization were excluded from the at-risk person-time. ICU denotes intensive care unit.

† The analysis was adjusted for age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19.

‡ A stratified version of the extended Cox proportional-hazards model was fit to test the robustness of the estimates to model assumptions, with stratification according to age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19.

death. Among fully immunized persons, the adjusted vaccine effectiveness was 65.9% for Covid-19 and 87.5% for hospitalization, 90.3% for ICU admission, and 86.3% for death. The vaccine-effectiveness results were maintained in both age-subgroup analyses, notably among persons 60 years of age or older, independent of variation in testing and independent of various factors regarding vaccine introduction in Chile.

The vaccine-effectiveness results in our study are similar to estimates that have been reported in Brazil for the prevention of Covid-19 (50.7%; 95% CI, 35.6 to 62.2), including estimates of cases that resulted in medical treatment (83.7%; 95% CI, 58.0 to 93.7) and estimates of a composite end point of hospitalized, severe, or fatal cases (100%; 95% CI, 56.4 to 100).[27] The large confidence intervals for the trial in Brazil reflect the relatively small sample (9823 participants) and the few cases detected (35 cases that led to medical treatment and 10 that were severe). However, our estimates are lower than the vaccine effectiveness recently reported in Turkey (83.5%; 95% CI, 65.4 to 92.1),[27,28] possibly owing to the small sample in that phase 3 clinical trial (10,029 participants in the per-protocol analysis), differences in local transmission dynamics, and the predominance of older adults among the fully or partially immunized participants in our study. Overall, our results suggest that the CoronaVac vaccine had high effectiveness against severe disease, hospitalizations, and death, findings that underscore the

Table 3. Effectiveness of CoronaVac Vaccine in Preventing Covid-19 Outcomes among Cohort Participants 60 Years of Age or Older, According to Immunization Status.

Outcome and Immunization Status	Subgroup Cohort	Persons with Covid-19		Vaccine Effectiveness (95% CI)		
	No. of Person-Days	No. of Persons	Incidence Rate	Analysis Adjusted for Sex and Age	Analysis Adjusted for All Covariates*	Stratified Analysis†
			no. of events/ 1000 person-days		*percent*	
Covid-19						
Unvaccinated	75,707,905	15,597	0.2060	—	—	—
Partially immunized	35,675,604	8,333	0.2336	3.9 (0.9–6.8)	9.7 (6.9–12.4)	12.7 (9.8–15.5)
Fully immunized	66,563,272	7,510	0.1128	63.4 (62.0–64.6)	66.6 (65.4–67.8)	67.2 (66.0–68.4)
Hospitalization						
Unvaccinated	76,047,640	5,304	0.0697	—	—	—
Partially immunized	35,961,593	2,168	0.0603	29.2 (25.1–33.1)	35.0 (31.3–38.6)	38.6 (34.8–42.2)
Fully immunized	66,986,859	1,344	0.0201	83.4 (82.2–84.5)	85.3 (84.3–86.3)	85.4 (84.3–86.4)
Admission to ICU						
Unvaccinated	76,194,648	1,811	0.0238	—	—	—
Partially immunized	36,062,081	672	0.0186	38.2 (31.9–44.0)	44.5 (38.7–49.7)	47.0 (41.2–52.2)
Fully immunized	67,051,769	331	0.0049	87.5 (85.7–89.0)	89.2 (87.6–90.6)	89.3 (87.8–90.7)
Confirmed death						
Unvaccinated	76,169,386	1,999	0.0262	—	—	—
Partially immunized	36,053,806	768	0.0213	39.7 (33.8–45.1)	45.8 (40.4–50.7)	46.1 (40.5–51.2)
Fully immunized	67,045,620	402	0.0060	84.4 (82.3–86.2)	86.5 (84.6–88.1)	86.8 (85.0–88.4)

* The analysis was adjusted for age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19.

† A stratified version of the extended Cox proportional-hazards model was fit to test the robustness of the estimates to model assumptions, with stratification according to sex, age, coexisting conditions, nationality, and income.

potential of this vaccine to save lives and substantially reduce demands on the health care system.

Our study has at least three main strengths. First, we used a rich administrative health care data set, combining data from an integrated vaccination system for the total population and from the Ministry of Health FONASA, which covers approximately 80% of the Chilean population. These data include information on laboratory tests, hospitalization, mortality, onset of symptoms, and clinical history in order to identify risk factors for severe disease. Information on region of residence also allowed us to control for differences in incidence across the country. We adjusted for income and nationality, which correlate with socioeconomic status in Chile and are thus considered to be social determinants of health. The large population sample allowed us to estimate vaccine effectiveness both for one dose and for the complete two-dose vaccination schedule. It also allowed for a subgroup analysis involving adults 60 years of age or older, a subgroup that is at higher risk for severe disease[3] and that is underrepresented in clinical trials. Second, data were collected during a rapid vaccination campaign with high uptake and during a period with one of the highest community transmission rates of the pandemic, which allowed for a relatively short follow-up period and for estimation of the prevention of at least four essential outcomes: Covid-19 cases and related hospitalization, ICU admission, and death. Finally, Chile has the highest testing rates for Covid-19 in Latin America, universal health care access, and a standardized, public reporting system for vital statistics, which limited the number of undetected or unascertained cases and deaths.[14]

Our study has several limitations. First, as an observational study, it is subject to confounding. To account for known confounders, we adjusted the analyses for relevant variables that could affect vaccine effectiveness, such as age, sex, underlying medical conditions, region of residence, and nationality. The risk of misclassification bias that would be due to the time-dependent performance of the SARS-CoV-2 RT-PCR assay is relatively low, because the median time from symptom onset to testing in Chile is approximately 4 days (98.1% of the tests were RT-PCR assays). In this 4-day period, the sensitivity and specificity of the molecular diagnosis of Covid-19 are high.[38] However, there may be a risk of selection bias. Systematic differences between the vaccinated and unvaccinated groups, such as health-seeking behavior or risk aversion, may affect the probability of exposure to the vaccine and the risk of Covid-19 and related outcomes.[39,40] However, we cannot be sure about the direction of the effect. Persons may be hesitant to get the vaccine for various reasons, including fear of side effects, lack of trust in the government or pharmaceutical companies, or an opinion that they do not need it, and they may be more or less risk-averse. Vaccinated persons may compensate by increasing their risky behavior (Peltzman effect).[40] We addressed potential differences in health care access by restricting the analysis to persons who had undergone diagnostic testing, and we found results that were consistent with those of our main analysis.

Second, owing to the relatively short follow-up in this study, late outcomes may not have yet developed in persons who were infected near the end of the study, because the time from symptom onset to hospitalization or death can vary substantially.[3,15] Therefore, effectiveness estimates regarding severe disease and death, in particular, should be interpreted with caution. Third, during the study period, ICUs in Chile were operating at 93.5% of their capacity on average (65.7% of the patients had Covid-19).[32] If fewer persons were hospitalized than would be under regular ICU operation, our effectiveness estimates for protection against ICU admission might be biased downward, and our effectiveness estimates for protection against death might be biased upward (e.g., if patients received care at a level lower than would usually be received during regular health system operation).

Fourth, although the national genomic surveillance for SARS-CoV-2 in Chile has reported the circulation of at least two viral lineages considered to be variants of concern, P.1 and B.1.1.7 (or the gamma and alpha variants, respectively),[41] we lack representative data to estimate their effect on vaccine effectiveness (Table S2). Results from a test-negative design study of the effectiveness of the CoronaVac vaccine in health care workers in Manaus, Brazil, where the gamma variant is now predominant, showed that the efficacy of at least one dose of the vaccine against Covid-19 was 49.6% (95% CI, 11.3 to 71.4).[30] Although the vaccine-effectiveness estimates in Brazil are not directly comparable with our estimates owing to differences in the target population, the vaccination schedule (a window of 14 to 28 days between doses is recommended in Brazil[42]), and immunization status, they highlight the importance of continued vaccine-effectiveness monitoring.

Overall, our study results suggest that the CoronaVac vaccine was highly effective in protecting against severe disease and death, findings that are consistent with the results of phase 2 trials[23,24] and with preliminary efficacy data.[27,28]

The research protocol was approved by the Comité Ético Científico Clínica Alemana Universidad del Desarrollo. The study was considered exempt from informed consent; no human health risks were identified. Research analysts are employees of the Chilean Ministry of Health; our use of data follows Chilean law 19.628 on private data protection.

Presented in part at the World Health Organization (WHO) Strategic Advisory Group of Experts on Immunization (SAGE) meeting on coronavirus disease 2019 (Covid-19) vaccines, April 29, 2021, and the WHO Vaccines Prioritization Working Group, April 21, 2021.

Supported by grants from the Agencia Nacional de Investigación y Desarrollo (ANID) Millennium Science Initiative Program, Millennium Nucleus Center for the Discovery of Structures in Complex Data (MIDAS) (NCN17_059, to Dr. Jara), and Millennium Initiative for Collaborative Research in Bacterial Resistance (MICROB-R) (NCN17_081, to Drs. Undurraga and Araos); the Advanced Center for Chronic Diseases, ANID Fondo de Financiamiento de Centros de Investigación en Áreas Prioritarias (FONDAP) (15130011, to Dr. Araos); and the Research Center for Integrated Disaster Risk Management ANID FONDAP (15110017, to Dr. Undurraga).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

Owing to data privacy regulations, the individual-level data in this study cannot be shared (Law N19.628). Aggregate data on vaccination and incidence are publicly available at https://github.com/MinCiencia/Datos-COVID19/.

We thank the attendees of the WHO meeting and working group for suggestions and comments; Drs. Ira Longini (University of Florida), Daniel Cohen (Tel Aviv University), Richard Peto (University of Oxford), Elizabeth Miller (Public Health England), and Myron M. Levine (University of Maryland); Drs. Catterina Ferreccio (Pontificia Universidad Católica de Chile), Ximena Aguilera (Universidad del Desarrollo), Maria T. Valenzuela (Universidad de Los Andes), Pablo Vial (Universidad del Desarrollo), Gonzalo Valdivia (Pontificia Universidad Católica de Chile), Fernando Otaiza (Ministerio de Salud), and Alvaro Erazo (Pontificia Universidad Católica de Chile), from the Chilean Covid-19 Advisory Group, for advice on the study design and interpretation of results; and Drs. José R. Zubizarreta (Harvard University) and Erika M.C. D'Agata (Brown University) for thoughtful suggestions.

REFERENCES

1. Dong E, Du H, Gardner L. An interactive Web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 2020;20:533-4.
2. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. JAMA 2020;324:782-93.
3. Verity R, Okell LC, Dorigatti I, et al. Estimates of the severity of coronavirus disease 2019: a model-based analysis. Lancet Infect Dis 2020;20:669-77.
4. Guan W-J, Ni Z-Y, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708-20.
5. Li Y, Campbell H, Kulkarni D, et al. The temporal association of introducing and lifting non-pharmaceutical interventions with the time-varying reproduction number (R) of SARS-CoV-2: a modelling study across 131 countries. Lancet Infect Dis 2021;21:193-202.
6. Walker PGT, Whittaker C, Watson OJ, et al. The impact of COVID-19 and strategies for mitigation and suppression in low- and middle-income countries. Science 2020;369:413-22.
7. Ledford H, Cyranoski D, Van Noorden R. The UK has approved a COVID vaccine — here's what scientists now want to know. Nature 2020;588:205-6.
8. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med 2020;383:2603-15.
9. Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet 2021;397:99-111.
10. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med 2021;384:403-16.
11. Logunov DY, Dolzhikova IV, Shcheblyakov DV, et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. Lancet 2021;397:671-81.
12. Tanne JH. Covid-19: FDA panel votes to approve Pfizer BioNTech vaccine. BMJ 2020;371:m4799.
13. Zimmer C, Corum J, Wee S-L. Coronavirus vaccine tracker. New York Times, June 10, 2021 (https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html).
14. Our World in Data. Coronavirus pandemic (COVID-19). 2021 (https://ourworldindata.org/coronavirus).
15. Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA Covid-19 vaccine in a nationwide mass vaccination setting. N Engl J Med 2021;384:1412-23.
16. Vasileiou E, Simpson CR, Robertson C, et al. Effectiveness of first dose of COVID-19 vaccines against hospital admissions in Scotland: national prospective cohort study of 5.4 million people. February 19, 2021 (https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3789264). preprint.
17. Thompson MG, Burgess JL, Naleway AL, et al. Interim estimates of vaccine effectiveness of BNT162b2 and mRNA-1273 COVID-19 vaccines in preventing SARS-CoV-2 infection among health care personnel, first responders, and other essential and frontline workers — eight U.S. locations, December 2020–March 2021. MMWR Morb Mortal Wkly Rep 2021;70:495-500.
18. Daniel W, Nivet M, Warner J, Podolsky DK. Early evidence of the effect of SARS-CoV-2 vaccine at one medical center. N Engl J Med 2021;384:1962-3.
19. Britton A, Jacobs Slifka KM, Edens C, et al. Effectiveness of the Pfizer-BioNTech COVID-19 vaccine among residents of two skilled nursing facilities experiencing COVID-19 outbreaks — Connecticut, December 2020–February 2021. MMWR Morb Mortal Wkly Rep 2021;70:396-401.
20. Doshi P. Will covid-19 vaccines save lives? Current trials aren't designed to tell us. BMJ 2020;371:m4037.
21. Lopalco PL, DeStefano F. The complementary roles of phase 3 trials and post-licensure surveillance in the evaluation of new vaccines. Vaccine 2015;33:1541-8.
22. Gao Q, Bao L, Mao H, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science 2020;369:77-81.
23. Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021;21:181-92.
24. Wu Z, Hu Y, Xu M, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis 2021;21:803-12.
25. Cohen J, Moutinho S. Third time's the charm? Brazil scales back efficacy claims for COVID-19 vaccine from China. Science. January 12, 2021 (https://www.sciencemag.org/news/2021/01/third-time-s-charm-brazil-scales-back-efficacy-claims-covid-19-vaccine-china).
26. Baraniuk C. What do we know about China's covid-19 vaccines? BMJ 2021;373:n912.
27. Sinovac. Summary of clinical trial data of Sinovac's COVID-19 vaccine (CoronaVac). 2021. (In Chinese) (http://www.sinovacbio.com/?optionid=754&auto_id=927).
28. Tanriover MD, Doğanay HL, Akova M, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. Lancet 2021 July 8 (Epub ahead of print).
29. Centers for Disease Control and Prevention. SARS-CoV-2 variant classifications and definitions. 2021 (https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Fcases-updates%2Fvariant-surveillance%2Fvariant-info.html).
30. Hitchings MDT, Ranzani OT, Scaramuzzini Torres MS, et al. Effectiveness of CoronaVac in the setting of high SARS-CoV-2 P.1 variant transmission in Brazil: a test-negative case-control study. May 1, 2021 (https://www.medrxiv.org/content/10.1101/2021.04.07.21255081v2). preprint.
31. Shepherd A. Covid-19: Chile joins top five countries in world vaccination league. BMJ 2021;372:n718.
32. Cifras Oficiales COVID-19. Ministerio de Salud, plan de acción coronavirus COVID-19, 2020 (https://www.gob.cl/coronavirus/cifrasoficiales/).
33. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. JAMA 2020;323:2052-9.
34. Bhargava A, Fukushima EA, Levine M, et al. Predictors for severe COVID-19 infection. Clin Infect Dis 2020;71:1962-8.
35. Numbers K, Brodaty H. The effects of the COVID-19 pandemic on people with dementia. Nat Rev Neurol 2021;17:69-70.
36. Therneau TM. A package for survival analysis in R: R package version 3.1-12. Rochester, MN: Mayo Clinic, April 2021 (https://cran.r-project.org/package=survival/survival.pdf).
37. R Development Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2019.
38. Jara A, Undurraga EA, Araos R. Tool for estimating the probability of having COVID-19 with one or more negative RT-PCR results. January 24, 2021 (https://www.medrxiv.org/content/10.1101/2021.01.16.21249939v1). preprint.
39. Lipsitch M, Jha A, Simonsen L. Observational studies and the difficult quest for causality: lessons from vaccine effectiveness and impact studies. Int J Epidemiol 2016;45:2060-74.
40. Trogen B, Caplan A. Risk compensation and COVID-19 vaccines. Ann Intern Med 2021;174:858-9.
41. Ministerio de Salud. Reporte circulación de variantes SARS-CoV-2 en Chile. Santiago, Chile: Departamento de Epidemiología Ministerio de Salud, 2021.
42. Ministério da Saúde. Informe técnico: campanha nacional de vacinação contra a Covid-19. January 19, 2021 (https://www.conasems.org.br/wp-content/uploads/2021/01/16110781637 93_Informe_Tecnico_da_Campanha_Nacional_de_Vacinacao_contra_a_Covid_19-1.pdf).

6.13. Mortalidade de crianças por Covid é muito maior em países pobres, onde vacinação dos mais novos não está prevista

A mortalidade de crianças por Covid-19 é muito maior em países pobres do que nos países ricos, ou seja, justamente nas nações que ainda não incluíram esse público em seus programas de vacinação. A desigualdade na distribuição de vacinas e no atendimento médico explicam o problema e abrem a discussão de quando e como incluir essa população na vacinação contra Covid-19, escreveram as pesquisadoras Beate Kampmann e Uduak Okomo, da London School of Hygiene &Tropical Medicine, em um artigo na revista científica The Lancet.

As pesquisadoras levantam a tese com base nos resultados de uma meta-análise (método estatístico que analisa dados de dois ou mais estudos) que concluiu que 91,5% das mortes globais de crianças e adolescentes por Covid-19 foram notificadas em países de baixa e média renda, enquanto 83,5% da população pediátrica infectada era proveniente destes países.

O robusto estudo, que revisou mais de 16 mil artigos científicos e 225 relatórios nacionais de 216 países, apontou que a taxa de mortalidade foi significativamente mais alta em países de baixa e média renda do que nos países ricos: 2,77 versus 1,32 a cada 1 milhão de crianças. Os dados compilados por pesquisadores da Universidade de Toronto foram publicados na revista científica PLOS One.

"Esta grande desigualdade impede que os países de baixa e média renda não apenas previnam a morte e doenças graves, mas também implantem vacinas como ferramentas para interromper a transmissão

do SARS-CoV-2. A inclusão das crianças e adolescentes não será uma prioridade nestes países mais pobres por um longo tempo por causa das graves deficiências na distribuição das vacinas", descrevem no artigo.

Diante dos dados, as pesquisadoras apontam que a proteção das crianças contra Covid-19 dependerá mais de fatores nacionais e de políticas públicas, que podem incluir ou não o acesso desse público às vacinas.

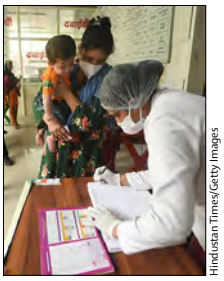
"Os impactos da vacinação contra Covid-19 em crianças e adolescentes na dinâmica de transmissão irão variar nacionalmente, levando em conta circunstâncias epidemiológicas, o surgimento de novas variantes do SARS-CoV-2 e estratégias de mitigação de contato com papéis diferentes em lugares diferentes", completam.

Tanta desigualdade desfoca os resultados de estudos com vacinas de vírus inativado, como a CoronaVac, e vacinas de RNA mensageiro, que demonstraram ser seguras e imunogênicas para crianças e adolescentes, na opinião das pesquisadoras.

"Não há razão para acreditar que as vacinas não devam ser igualmente protetoras contra Covid-19 em crianças e adolescentes, como nos adultos. Mais de 30 ensaios internacionais recrutam crianças e adolescentes a partir de seis meses para avaliar a segurança, imunogenicidade, dosagem e distribuição", explicam.

Publicado em: 30/7/2021

COVID-19 vaccines for children in LMICs: another equity issue



Hindustan Times/Getty Images

Published Online
July 30, 2021
https://doi.org/10.1016/
S0140-6736(21)01748-7

Given the success of COVID-19 vaccines in preventing death and severe disease in adults[1] and their impact on community transmission,[2] use in children and young people (CYP) inevitably requires consideration. Although severe COVID-19 is rare in CYP,[3] they are affected by SARS-CoV-2 infection and the impacts of the COVID-19 pandemic, including education, mental health, and general wellbeing.[4]

As of late July, 2021, no COVID-19 vaccine is recommended for children younger than 12 years and safety and efficacy data from phase 3 clinical trials are so far limited: 1131 CYP aged 12–15 years received the Pfizer–BioNTech mRNA vaccine[5] and safety data are available from phase 1 and 2 trials of Sinovac's inactivated CoronaVac vaccine in 438 children aged 3–17 years.[6] Safety data have been reassuring, with published data confirming excellent immunogenicity.[5] There is no reason to believe the vaccines should not be equally protective against COVID-19 in CYP as they are in adults. More than 30 international trials are now recruiting CYP as young as 6 months to assess safety, immunogenicity, dosing, and scheduling questions.[7] Safety data from the Pfizer–BioNTech mRNA vaccine trial proved sufficient for regulatory authorities in the EU, Israel, and North America to issue approval for use of this vaccine in CYP aged 12–15 years. Safety data from the real-life roll-out of COVID-19 vaccines are continuously collected through surveillance systems in high-income countries (HICs)[8,9] and are generally reassuring, although a rare vaccine-associated signal of transient inflammation of the heart muscle in some young adults has raised concerns.[10] On balance, the US Centers for Disease Control and Prevention concluded that benefits outweigh the risks.[11]

Countries are also still calculating what indirect benefits for reduced SARS-CoV-2 transmission in schools and the wider community could be achieved by vaccinating CYP. With children now recognised as part of the chains of community transmission,[4] the discussion about a CYP vaccine programme was perhaps inescapable. Yet the impacts of COVID-19 vaccination in CYP on transmission dynamics will vary nationally, since epidemiological circumstances, novel SARS-CoV-2 variants, and contact mitigation strategies will have different roles in different places.

Most countries have yet to decide whether to include CYP in COVID-19 vaccination programmes. Canada, Israel, some European countries, and the USA have introduced the vaccine for all young people older than 12 years. By contrast, countries such as Germany and the UK are focusing on groups most at risk of severe COVID-19, but are not universally rolling out COVID-19 vaccination to CYP older than 12 years.[12]

Unsurprisingly, low-income and middle-income countries (LMICs) have not yet introduced COVID-19 vaccines for CYP. WHO guidance from July 14, 2021, states: "Children and adolescents tend to have milder disease compared to adults, so unless they are part of a group at higher risk of severe COVID-19, it is less urgent to vaccinate them than older people, those with chronic health conditions and health workers…WHO's Strategic Advisory Group of Experts (SAGE) has concluded that the Pfizer–BioNTech vaccine is suitable for use by people aged 12 years and above. Children aged between 12 and 15 who are at high risk may be offered this vaccine alongside other priority groups for vaccination. Vaccine trials for children are ongoing and WHO will update its recommendations when the evidence or epidemiological situation warrants a change in policy."[13]

Further data from LMICs will aid risk assessments of SARS-CoV-2 in CYP, both for personal health and transmission roles. A recent meta-analysis indicated that the outcome of children admitted to hospital with acute COVID-19 is worse in LMICs than in HICs (case fatality rates 0·29% [95% CI 0·28–0·31%] *vs* 0·03% [0·03–0·03%]).[14] Vaccinating CYP in LMICs may ultimately have more benefit to their health status compared with CYP in HICs.

All vaccines should be given to those who need them most, particularly in the context of a pandemic with limited vaccine supply. Of the more than 4 billion doses of COVID-19 vaccines administered globally in the past 8 months, less than 2% have been given in Africa;[15] on a continent that cannot vaccinate its most vulnerable populations (eg, older people and those with chronic conditions) and highly exposed health-care workers, introducing vaccines for CYP remains a luxury. This gross inequity prevents LMICs from not only preventing death and serious illness,

ɔmment

but also from deploying vaccines as tools to interrupt SARS-CoV-2 transmission. The inclusion of CYP will not be a priority in LMICs for a long time because of the serious shortfalls of vaccines.

What of the WHO motto that "No one is safe till everyone is safe"? HICs have unlimited stocks of COVID-19 vaccines.[16] If a key reason for the use of the COVID-19 vaccines in CYP in HICs is reducing SARS-CoV-2 transmission, surely CYP in LMICs should also be vaccinated? We are far from the vision of the African Union (AU) to vaccinate two-thirds of its members' population. In addition to COVAX, the AU has now partnered with additional vaccine suppliers through the AU's African Vaccine Acquisition Trust, including UNICEF.[17] However, even vaccinating 66% of individuals is unlikely to be sufficient to interrupt transmission chains.

In addition to supply issues and logistics that prevent the use of COVID-19 vaccines in CYP in LMICs, the success of any plans to roll out the vaccines must also ride on the back of acceptance and confidence. Parents in LMICs need reassurance they are doing the right thing for their children, just as has been found in HICs.[18]

During deliberations on the potential benefits of COVID-19 vaccines for CYP, it is important to recognise that this pandemic has already deprived more than 8 million children, primarily in LMICs, from life-saving, routine childhood vaccines.[19] Immunisation services are preoccupied with the implementation of COVID-19 vaccine programmes for adults. At present, greater benefit for children's health globally will be derived by delivering the health interventions we already know will save their lives, such as vaccines against measles and other vaccine-preventable diseases, than by focusing on delivering COVID-19 vaccines to part of a population that does not currently represent a strategic priority in the response to this pandemic. Although maybe not equitable, we believe this approach is more important for the health of CYP at this point in time.

BK has received institutional grants from Pfizer for a maternal immunisation study unrelated to COVID-19; and received personal fees for services to a Data and Safety Monitoring Board from Johnson & Johnson for a COVID-19 vaccine study and for scientific advisory boards from Pfizer, Sanofi, and GlaxoSmithKline for non-COVID-19-related vaccines for use in pregnancy. UO declares no competing interests.

Beate Kampmann, Uduak Okomo
beate.kampmann@lshtm.ac.uk

The Vaccine Centre, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK (BK); Vaccines and Immunity Theme, MRC Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, Fajara, The Gambia (BK, UO)

1 Haas EJ, Angulo FJ, McLaughlin JM, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. *Lancet* 2021; **397:** 1819–29.
2 Harris RJ, Hall JA, Zaidi A, et al. Effect of vaccination on household transmission of SARS-CoV2 in England. *N Engl J Med* 2021; published online June 23. https://doi.org/10.1056/NEJMc2107717.
3 Irfan O, Muttalib F, Tang K, et al. Clinical characteristics, treatment and outcomes of paediatric COVID-19: a systematic review and meta-analysis. *Arch Dis Child* 2021; **106:** 440–48.
4 Ismail SA, Saliba V, Bernal JL, et al. SARS-CoV-2 infection and transmission in educational settings: a prospective, cross-sectional analysis of infection clusters and outbreaks in England. *Lancet Infect Dis* 2021; **21:** 344–53.
5 Frenck Jr RW, Klein NP, Kitchin N, et al. Safety, immunogenicity, and efficacy of the BNT162b2 Covid-19 vaccine in adolescents. *N Engl J Med* 2021; **385:** 239–50.
6 Han B, Song Y, Li C, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; published online June 28. https://doi.org/10.1016/S1473-3099(21)00319-4.
7 London School of Hygiene & Tropical Medicine. LSHTM Vaccine Tracker. 2021. https://vac-lshtm.shinyapps.io/ncov_vaccine_landscape/ (accessed July 28, 2021).
8 Medicines and Healthcare products Regulatory Agency. MHRA Coronavirus Yellow Card reporting. 2021. https://coronavirus-yellowcard.mhra.gov.uk (accessed July 28, 2021).
9 US Centers for Disease Control and Prevention. Vaccine Adverse Event Reporting System (VAERS). 2021. https://www.cdc.gov/vaccinesafety/ensuringsafety/monitoring/vaers/index.html (accessed July 28, 2021).
10 Montgomery J, Ryan M, Engler R, et al. Myocarditis following immunization with mRNA COVID-19 vaccines in members of the US military. *JAMA Cardiol* 2021; published online June 29. https://doi.org/10.1001/jamacardio.2021.2833.
11 Gargano JW, Wallace M, Hadler SC, et al. Use of mRNA COVID-19 vaccine after reports of myocarditis among vaccine recipients: update from the Advisory Committee on Immunization Practices—United States, June 2021. *MMWR Morb Mortal Wkly Rep* 2021; **70:** 977–82.
12 Public Health England. JCVI issues advice on COVID-19 vaccination of children and young people. July 19, 2021. https://www.gov.uk/government/news/jcvi-issues-advice-on-covid-19-vaccination-of-children-and-young-people (accessed July 28, 2021).
13 WHO. COVID-19 advice for the public: getting vaccinated. July 14, 2021. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines/advice (accessed July 28, 2021).
14 Kitano T, Kitano M, Krueger C, et al. The differential impact of pediatric COVID-19 between high-income countries and low- and middle-income countries: a systematic review of fatality and ICU admission in children worldwide. *PLoS One* 2021; **16:** e0246326.
15 African Union. COVID-19 vaccination—latest updates from Africa CDC on progress made in COVID-19 vaccinations on the continent. 2021. https://africacdc.org/covid-19-vaccination/ (accessed July 28, 2021).
16 Wouters O J, Shadlen K C, Salcher-Konrad M, et al. Challenges in ensuring global access to COVID-19 vaccines: production, affordability, allocation, and deployment. *Lancet* 2021; **397:** 1023–34.
17 UNICEF. The African Union's African Vaccine Acquisition Trust (AVAT) initiative. 2021. https://www.unicef.org/supply/african-unions-african-vaccine-acquisition-trust-avat-initiative (accessed July 28, 2021).
18 Bell S, Clarke R, Mounier-Jack S, et al. Parents' and guardians' views on the acceptability of a future COVID-19 vaccine: a multi-methods study in England. *Vaccine* 2020; **38:** 7789–98.
19 Causey K, Fullman N, Sorensen RJD, et al. Estimating global and regional disruptions to routine childhood vaccine coverage during the COVID-19 pandemic in 2020: a modelling study. *Lancet* 2021; published online July 14. https://doi.org/10.1016/S0140-6736(21)01337-4.

6.14. CoronaVac é segura e gera forte resposta imune em crianças e adolescentes, confirma estudo

A CoronaVac, vacina contra a Covid-19 desenvolvida pela biofarmacêutica chinesa Sinovac Biotech e produzida no Brasil pelo Butantan, é segura para a população de três a 17 anos de idade e pode induzir uma forte produção de anticorpos no grupo pediátrico. As conclusões foram obtidas nos estudos clínicos de fases 1 e 2 conduzidos pela Sinovac com a aplicação da CoronaVac em crianças e adolescentes. Os resultados foram publicados no periódico científico The Lancet Infectious Diseases.

Este é o primeiro estudo do mundo a avaliar o uso de uma vacina contra a Covid-19 em uma população a partir dos três anos de idade. "Crianças e adolescentes com Covid-19 geralmente têm infecções leves ou assintomáticas em comparação aos adultos. Apesar disso, um pequeno número ainda pode estar em risco de doença grave e essa população ainda pode transmitir o vírus a outras pessoas. Portanto, é vital testar a segurança e a eficácia das vacinas contra a Covid-19 em grupos de idades mais jovens", disse o gerente geral da Sinovac, Gao Qiang, em comunicado publicado no site da farmacêutica.

O estudo randomizado, controlado e duplo-cego avaliou 550 crianças (71 na fase 1 e 479 na fase 2) de três a 17 anos para medir a segurança, a tolerabilidade e a imunogenicidade da aplicação de duas doses da Coronavac com um intervalo de 28 dias entre elas.

Um grupo tomou a vacina enquanto o outro recebeu placebo com hidróxido de alumínio, adjuvante não nocivo ao organismo presente na fórmula do imunizante. As análises apontaram que a vacina foi capaz de gerar anticorpos em 96% dos voluntários 28 dias após a segunda dose. Na fase 1, nenhum dos participantes tinha anticorpos neutralizantes contra o SARS-CoV-2 e, 28 dias após a vacinação, 100% deles apresentaram anticorpos.

Na fase 2, alguns voluntários receberam duas aplicações com dosagens menores (1,5μg) e outros receberam dosagens maiores (3μg). Enquanto no primeiro grupo 95% dos participantes apresentaram anticorpos no sangue, este número foi de 100% no segundo grupo. Por isso, os pesquisadores optaram por seguir apenas com a dosagem mais alta no ensaio clínico de fase 3, que ainda está em andamento.

As reações adversas foram de leves a moderadas, sendo dor no local da aplicação e febre as mais comuns, com desaparecimento dos sintomas em até 48 horas. 27% dos participantes relataram efeitos colaterais. Houve apenas um caso de evento adverso grave, não associado à vacina - uma criança teve pneumonia após receber placebo.

Publicado em: 28/06/2021

Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial

Bihua Han, Yufei Song*, Changgui Li*, Wanqi Yang, Qingxia Ma, Zhiwei Jiang, Minjie Li, Xiaojuan Lian, Wenbin Jiao, Lei Wang, Qun Shu, Zhiwei Wu, Yuliang Zhao, Qi Li, Qiang Gao*

Summary

Background A vaccine against SARS-CoV-2 for children and adolescents will play an important role in curbing the COVID-19 pandemic. Here we aimed to assess the safety, tolerability, and immunogenicity of a candidate COVID-19 vaccine, CoronaVac, containing inactivated SARS-CoV-2, in children and adolescents aged 3–17 years.

Methods We did a double-blind, randomised, controlled, phase 1/2 clinical trial of CoronaVac in healthy children and adolescents aged 3–17 years old at Hebei Provincial Center for Disease Control and Prevention in Zanhuang (Hebei, China). Individuals with SARS-CoV-2 exposure or infection history were excluded. Vaccine (in 0·5 mL aluminum hydroxide adjuvant) or aluminum hydroxide only (alum only, control) was given by intramuscular injection in two doses (day 0 and day 28). We did a phase 1 trial in 72 participants with an age de-escalation in three groups and dose-escalation in two blocks (1·5 μg or 3·0 μg per injection). Within each block, participants were randomly assigned (3:1) by means of block randomisation to receive CoronaVac or alum only. In phase 2, participants were randomly assigned (2:2:1) by means of block randomisation to receive either CoronaVac at 1·5 μg or 3·0 μg per dose, or alum only. All participants, investigators, and laboratory staff were masked to group allocation. The primary safety endpoint was adverse reactions within 28 days after each injection in all participants who received at least one dose. The primary immunogenicity endpoint assessed in the per-protocol population was seroconversion rate of neutralising antibody to live SARS-CoV-2 at 28 days after the second injection. This study is ongoing and is registered with ClinicalTrials.gov, NCT04551547.

Findings Between Oct 31, 2020, and Dec 2, 2020, 72 participants were enrolled in phase 1, and between Dec 12, 2020, and Dec 30, 2020, 480 participants were enrolled in phase 2. 550 participants received at least one dose of vaccine or alum only (n=71 for phase 1 and n=479 for phase 2; safety population). In the combined safety profile of phase 1 and phase 2, any adverse reactions within 28 days after injection occurred in 56 (26%) of 219 participants in the 1·5 μg group, 63 (29%) of 217 in the 3·0 μg group, and 27 (24%) of 114 in the alum-only group, without significant difference (p=0·55). Most adverse reactions were mild and moderate in severity. Injection site pain was the most frequently reported event (73 [13%] of 550 participants), occurring in 36 (16%) of 219 participants in the 1·5 μg group, 35 (16%) of 217 in the 3·0 μg group, and two (2%) in the alum-only group. As of June 12, 2021, only one serious adverse event of pneumonia has been reported in the alum-only group, which was considered unrelated to vaccination. In phase 1, seroconversion of neutralising antibody after the second dose was observed in 27 of 27 participants (100·0% [95% CI 87·2–100·0]) in the 1·5 μg group and 26 of 26 participants (100·0% [86·8-100·0]) in the 3·0 μg group, with the geometric mean titres of 55·0 (95% CI 38·9–77·9) and 117·4 (87·8–157·0). In phase 2, seroconversion was seen in 180 of 186 participants (96·8% [93·1–98·8]) in the 1·5 μg group and 180 of 180 participants (100·0% [98·0–100·0]) in the 3·0 μg group, with the geometric mean titres of 86·4 (73·9–101·0) and 142·2 (124·7–162·1). There were no detectable antibody responses in the alum-only groups.

Interpretation CoronaVac was well tolerated and safe and induced humoral responses in children and adolescents aged 3–17 years. Neutralising antibody titres induced by the 3·0 μg dose were higher than those of the 1·5 μg dose. The results support the use of 3·0 μg dose with a two-immunisation schedule for further studies in children and adolescents.

Funding The Chinese National Key Research and Development Program and the Beijing Science and Technology Program.

Lancet Infect Dis 2021; 21: 1645–53

Published Online June 28, 2021 https://doi.org/10.1016/S1473-3099(21)00319-4

See Comment page 1614

*Contributed equally to the manuscript

Hebei Provincial Center for Disease Control and Prevention, Shijiazhuang, Hebei Province, China (B Han MSc, M Li MSc, Z Wu MSc, Y Zhao MSc, Qi Li PhD); **Sinovac Biotech, Beijing, China** (Y Song BSc, W Yang MSc, L Wang MSc); **National Institutes for Food and Drug Control, Beijing, China** (C Li PhD); **Zanhuang County Center for Disease Control and Prevention, Zanhuang, Hebei Province, China** (Q Ma BSc, W Jiao BSc); **Beijing Key Tech Statistics Technology, Beijing, China** (Z Jiang PhD, Q Shu MSc); **Sinovac Life Sciences, Beijing, China** (X Lian MBA, Q Gao MSc)

Correspondence to:
Dr Qiang Gao, Sinovac Life Sciences, Beijing, China
gaoq@sinovac.com

or

Dr Qi Li, Hebei Provincial Center for Disease Control and Prevention, Shijiazhuang, Hebei Province, China
liqicdc226@163.com

Introduction

The ongoing COVID-19 pandemic, caused by SARS-CoV-2, has led to more than 174·5 million infections and more than 3·8 million deaths worldwide as of June 11, 2021.[1] Children and adolescents infected with SARS-CoV-2 are mainly mild or asymptomatic compared with adults, but a

Research in context

Evidence before this study

We searched PubMed on Apr 29, 2021, for published research articles, with no language or date restrictions, using the search terms of "SARS-CoV-2", "COVID-19", "vaccine", and "clinical trial". We identified several clinical trials of COVID-19 vaccines across different platforms, including mRNA, viral vector, protein subunit, and inactivated virus. The results from phase 1–3 studies have confirmed that different vaccines were safe, effective, and induced humoral antibody responses in adults. As of April 19, 2020, more than ten COVID-19 candidate vaccines have been rolled out in many countries for general population use. Although vaccine companies have started to assess the safety and efficacy of COVID-19 vaccines in populations of 6 months to 17 years of age, there are currently no authorised vaccines for use among children and adolescents under the age of 16. We previously assessed CoronaVac, an inactivated vaccine developed by Sinovac Life Sciences, in adults aged 18–59 years and those aged 60 years and older, and showed that it was safe and well tolerated. Seroconversion rates ranged from 92% to 100% after two doses of CoronaVac (3·0 μg and 6·0 μg) with two immunisation schedules (on days 0 and 14, or on days 0 and 28) in adults aged 18–59 years. Seroconversion rates were higher than 98% after two doses of CoronaVac (3 μg and 6 μg) with the 0–28 days schedule in patients aged 60 years and older.

Added value of this study

This is, we believe, the first report of an inactivated SARS-CoV-2 vaccine, CoronaVac, tested in children and adolescents aged 3–17 years. CoronaVac was found to be well tolerated and safe in this population. The seroconversion rates of neutralising antibody with both doses (1·5 μg and 3·0 μg) were over 96% after two-dose vaccination and the neutralising antibody titres induced by the 3·0 μg dose were higher than those induced by the 1.5 μg dose. Taken together, the 3·0 μg dose of CoronaVac induced higher immune responses compared with 1·5 μg dose.

Implications of all the available evidence

While a small number of children and adolescents with SARS-CoV-2 infection might be at risk for severe COVID-19 and complicated illnesses, they usually have mild or asymptomatic symptoms compared with adults. Nevertheless, children and adolescents can be important transmitters of SARS-CoV-2 in communities. Therefore, testing the effectiveness of COVID-19 vaccines in this population is important. CoronaVac was well tolerated and immunogenic in healthy children and adolescents aged 3–17 years in this trial, which supports the use of CoronaVac for further studies in this population.

relatively small number of children and adolescents might be at risk for severe COVID-19, especially those with underlying health comorbidities.[2–5] Studies have also found that the SARS-CoV-2 infection can lead to a serious complication called multisystem inflammatory syndrome in children, which includes myocardial dysfunction, shock, and respiratory failure requiring intensive care.[3,6,7] Furthermore, children and adolescents can be important transmitters of SARS-CoV-2 in communities.[8,9] Therefore, testing the effectiveness of COVID-19 vaccines in this population is important. As of June 11, 2021, a total of 287 candidate vaccines are in clinical or preclinical development.[10] The results from phase 3 trials of multiple vaccines across three platforms, including mRNA, viral vector, and inactivated virus, have confirmed that the vaccines are effective in preventing SARS-CoV-2 infection in adults,[11,12] and more than ten vaccines have been rolled out in many countries for general population use. No COVID-19 vaccines are authorised for use among children under the age of 12 years, but vaccine companies have been started to assess the safety and efficacy of various vaccine platforms among the population aged 6 months to 17 years.[13,14] The mRNA vaccine developed by Pfizer has shown 100% efficacy and robust antibody responses in adolescents aged 12–15 years.[15]

Purified inactivated viruses have traditionally been used for vaccine development. CoronaVac is an inactivated SARS-CoV-2 vaccine developed by Sinovac Life Sciences (Beijing, China), which provided partial or complete protection in macaques following SARS-CoV-2 challenge, without observable antibody-dependent enhancement of infection.[16] The analyses from phase 1–3 trials have shown that CoronaVac was effective, immunogenic, and safe in adults aged 18 years and older.[12,17–19] Furthermore, another 11 inactivated COVID-19 candidate vaccines are in clinical evaluation, and several studies have also shown that the inactivated vaccines can induce neutralising antibody responses and have good safety profiles.[20–24]

The phase 1/2 trial of CoronaVac in children and adolescents was launched in October, 2020 to assess the safety, tolerability, and immunogenicity. Here we report the results of CoronaVac among healthy participants aged 3–17 years old.

Method

Study design and participants

We have done two phase 1/2 clinical trials of CoronaVac in participants aged 18–59 years and aged 60 years and older.[17,18] The preliminary immunogenicity and safety results supported the expansion of the trial to children and adolescents. We subsequently did a single-centre, randomised, double-blind, controlled, phase 1/2 trial to evaluate the safety, tolerability, and immunogenicity of CoronaVac in children and adolescents aged 3–17 years. On the basis of the results of previous trials and considering the low weight of this population, two different doses—1·5 μg and 3·0 μg—were adopted in this study.

This trial was run at Hebei Provincial Center for Disease Control and Prevention in Zanhuang (Hebei, China).

The phase 1 trial was an age de-escalation and dose-escalation study of 72 participants. Participants in each age group (3–5 years, 6–11 years, and 12–17 years) were recruited in order from the low-dose stage (block 1) to the high-dose stage (block 2). In block 1, participants were randomly assigned to receive either 1·5 μg vaccine or aluminum hydroxide adjuvant only (alum only, control) and participants in block 2 were randomly assigned to receive either 3·0 μg vaccine or alum only. In phase 1, 7 days of follow-up for safety were required before entering the next stage. The phase 2 trial was initiated only after all the participants in phase 1 had finished and passed a 7-days safety observation period after the first dose, as confirmed by the data monitoring committee. The required safety criteria were: no-life threatening vaccine-related adverse events (adverse reactions), no more than 15% of vaccinated participants reporting severe adverse reactions, and no other safety concerns in the opinion of the data monitoring committee. A total of 480 participants were recruited in phase 2, including 120 aged 3–5 years, 180 aged 6–11 years, and 180 aged 12–17 years.

Eligible participants were healthy children and adolescents aged 3–17 years. The key exclusion criteria included high-risk epidemiology history within 14 days before enrolment (eg, travel or residence history in communities with case reports, or contact history with someone infected with SARS-CoV-2), history of severe acute respiratory syndrome or SARS-CoV-2 infection (as reported by participants), axillary temperature of more than 37·0°, and history of allergy to any vaccine component. A complete list of exclusion criteria is listed in the protocol, which is available online.

Parents provided written informed consents, and participants 8–17 years of age also provided written assents before enrolment. The clinical trial protocol and informed consent form were approved by the Ethics Committee of Hebei CDC (IRB2020-005). The study was done in accordance with the requirements of Good Clinical Practice of China and the International Conference on Harmonisation.

Randomisation and masking

In phase 1, participants of block 1 and block 2 were randomly assigned (3:1) to either vaccine or alum only, and in phase 2, participants were randomly assigned (2:2:1) to either 1·5 μg, 3·0 μg of vaccine, or alum only. The randomisation codes for the phase 1 and phase 2 were generated by the randomisation statistician by means of block randomisation using SAS software (version 9.4). The randomisation code was assigned to each participant in sequence in the order of enrolment, and then the participants received the study vaccine labelled with the same code. The vaccine and alum only were completely identical in appearance, and all participants, investigators, and laboratory staff were masked to group allocation.

Procedures

CoronaVac is an inactivated vaccine candidate against SARS-CoV-2 infection. To prepare the vaccine, SARS-CoV-2 (CN02 strain) was propagated in African green monkey kidney cells (WHO Vero 10-87 Cells). At the end of the incubation period, the virus was harvested, inactivated with β-propiolactone, concentrated, purified, and finally adsorbed onto aluminum hydroxide. The aluminium hydroxide complex was then diluted in sodium chloride, phosphate-buffered saline, and water, before being sterilised and filtered for injection. The control was aluminum hydroxide adjuvant (alum only) with no virus. Both the vaccine and alum only were prepared in the Good Manufacturing Practice-accredited facility of Sinovac Life Science that was periodically inspected by the National Medical Products Administration committee for compliance. The production process of the vaccine in this trial was a highly automated bioreactor (ReadyToProcess WAVE 25, GE, Umea, Sweden), which was consistent with the production process of vaccine used in the phase 2 trial of adults aged 18–59 years and in the phase 1/2 trial of older adults aged at least 60 years.[17,18] Vaccine doses of 1·5 μg, or 3·0 μg in 0·5 mL of aluminium hydroxide diluent per dose and alum only in ready-to-use syringes were administered intramuscularly to participants on day 0 and day 28.

Participants were observed in the study site for at least 30 min after vaccination. For the first 7 days after each dose, parents or guardians of participants were required to record any injection-site adverse events (eg, pain, swelling, erythema), or systemic adverse events (eg, allergic reaction, cough, fever) on the diary cards. From day 8 to day 28 after each dose, safety data were collected by spontaneous report from the participants combined with the regular visit (which occurred on day 3, day 8 and day 28 after each dose in phase 1, and on day 8 and day 28 in phase 2). Solicited adverse events were recorded for 7 days after each dose and unsolicited adverse events for 28 days. The serious adverse events are recorded throughout the study and follow-up will continue until 12 months after the second dose. The reported adverse events were graded according to the China National Medical Products Administration guidelines.[25] The causal relationship between adverse events and vaccination was established by the investigators.

In the phase 1 trial, blood and urine samples were taken on day 3 after each dose and tested to investigate any abnormal changes of the haematology, biochemistry, and urine routine indexes. Blood samples were collected on day 0, 28, and 56 from participants in phase 1, and on day 0 and 56 in phase 2 to evaluate the neutralising antibody titres. The neutralising antibody titres to

For more on **exclusion criteria** see http://www.hebeicdc.cn/kygz/25011.jhtml

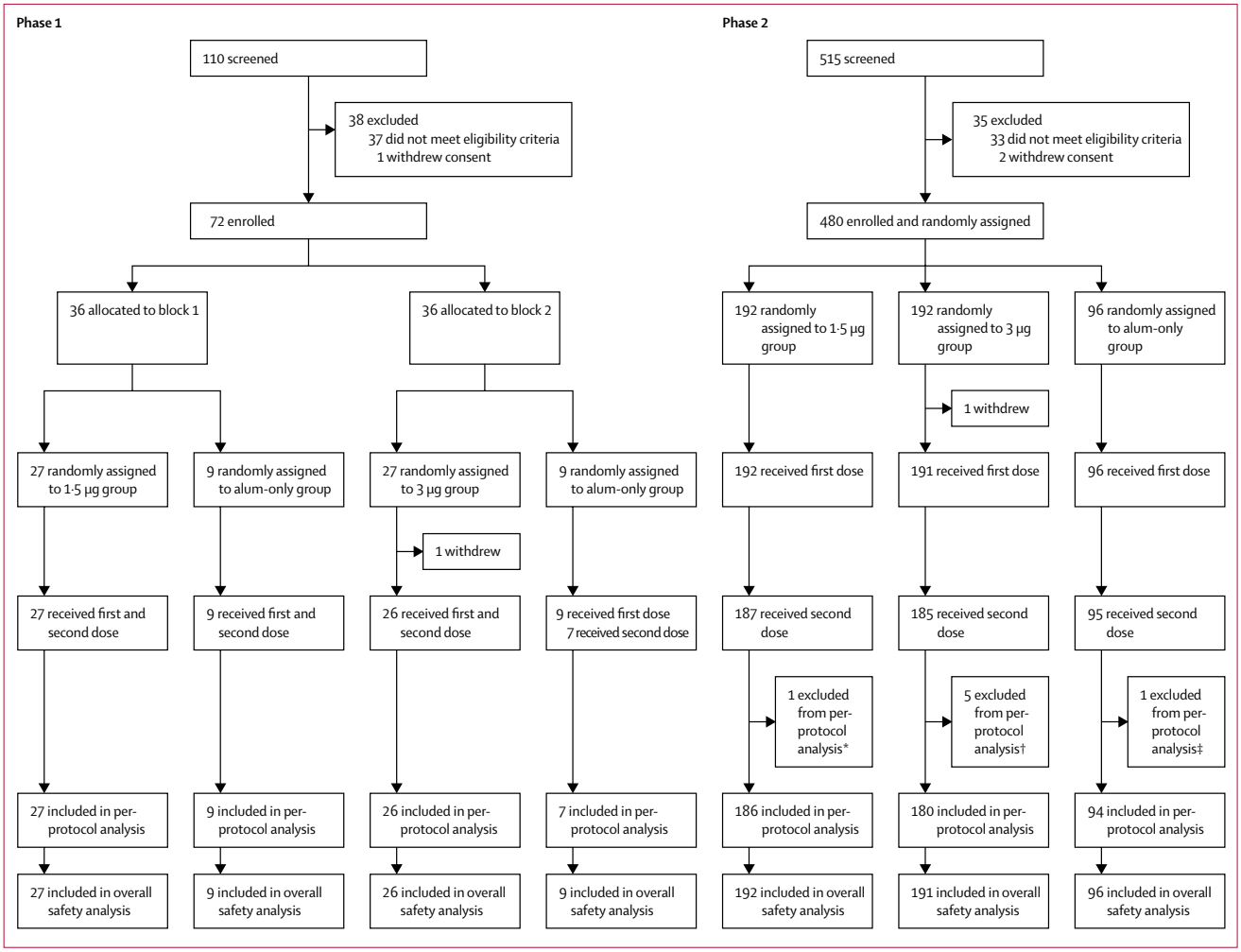


Figure 1: **Trial profile**

*One participant in the 1·5 µg group was excluded from the per-protocol analysis because he received tetanus immunoglobulin at day 14 after the second dose. †One participant in the 3 µg group was excluded from the per-protocol analysis because blood collection after vaccination was outside of the specified time window, and four did not have a blood sample taken 28 days after the second dose. ‡One participant in the alum only group was excluded from the per-protocol analysis because he did not have a blood sample taken 28 days after the second dose.

See Online for appendix

live SARS-CoV-2 (virus strain: SARS-CoV-2/human/CHN/CN1/2020, genebank number MT407649.1) was quantified by means of the microcytopathogenic effect assay.[26] Serum samples were inactivated at 56° for 30 min and serially diluted with cell culture medium in two-fold steps. The diluted serum samples were incubated with equal volume (50 µL) of the live SARS-CoV-2 virus suspension, with a 50% cell culture infective dose of 100 for 2 h at 37·0°. Vero cells (1·0–2·0×10^5 cells per mL) were then added to the serum–virus suspensions in microplates in duplicate and incubated at 36·5° for 5 days. Cytopathic effects were recorded under microscopes and the neutralising antibody titre was calculated by the dilution number of 50% protective condition. Detection was done by the National Institute for Food and Drug Control. Further information on the method has been provided in the appendix (p 1).

Outcomes

The primary safety endpoint was any vaccine-related adverse events (adverse reactions) within 28 days after the administration of each dose of the study vaccine or alum only. Secondary safety endpoints were serious adverse events and any abnormal changes in laboratory measurements at day 3 after each dose. Laboratory index tests were prespecified only in the phase 1 trial. The primary immunogenic endpoint was the seroconversion rate of neutralising antibodies to live SARS-CoV-2 at day 28 after the second dose. Secondary immunogenic endpoints were geometric mean titre (GMT) of neutralising antibodies to

live SARS-CoV-2, as well as seropositive rates and geometric mean increase. Seroconversion was defined as a change from seronegative at baseline to seropositive or a four-fold titre increase if the participant was seropositive at baseline. The positive cutoff of the titre for neutralising antibodies to live SARS-CoV-2 was 1/8.

Statistical analysis

We assessed the safety endpoints in the safety population, which included all participants who had received at least one dose of vaccine or alum only. We assessed the immunogenicity endpoints in the per-protocol population, which included all participants who had randomly received two doses of vaccine or alum only, had antibody results available, and did not violate the trial protocol.

We did not determine the sample sizes on the basis of a statistical power calculation, but followed the requirements of the China National Medical Products Administration and Chinese Technical Guidelines for Clinical Trials of Vaccines—ie, recruitment of at least 20–30 participants in phase 1 and 300 participants in phase 2 trial.

We used the Pearson χ^2 test or Fisher's exact test for the analysis of categorical outcomes. We calculated the 95% CIs for all categorical outcomes using the Clopper-Pearson method. We calculated GMTs and corresponding 95% CIs on the basis of the standard normal distribution of the log-transformation antibody titre. We used the ANOVA method to compare the log-transformed antibody titres. When the comparison among all groups showed significant difference, we then did pairwise comparisons. Hypothesis testing was two-sided and we considered a p value of less than 0·05 to be significant.

An independent data monitoring committee consisting of one independent statistician, one clinician, and one epidemiologist was established before commencement of the study. Safety data were assessed and reviewed by the committee to ensure further proceeding of the study. We used SAS (version 9.4) for all analyses. This trial is registered with ClinicalTrials.gov, NCT04551547.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Employees of Sinovac Life Sciences and Sinovac Biotech, listed as the authors, contributed to the study design, data interpretation, clinical trial monitoring, writing or revising the manuscript.

Results

Between Oct 31, 2020, and Dec 2, 2020, 110 individuals were screened and 72 were enrolled in phase 1. Between Dec 12 and Dec 30, 2020, 515 individuals were screened and 480 were enrolled in phase 2. 550 (>99%) of

	Phase 1			Phase 2		
	1·5 μg group (n=27)	3 μg group (n=26)	Aluminium hydroxide only group (n=18)	1·5 μg group (n=192)	3·0 μg group (n=191)	Aluminium hydroxide only group (n=96)
Age, years	8·4 (4·2)	8·2 (4·0)	8·3 (4·0)	9·3 (3·9)	9·2 (3·8)	9·1 (4·0)
3–5	9 (33%)	9 (35%)	6 (33%)	48 (25%)	47 (25%)	24 (25%)
6–11	9 (33%)	9 (35%)	6 (33%)	72 (38%)	72 (38%)	36 (38%)
12–17	9 (33%)	8 (31%)	6 (33%)	72 (38%)	72 (38%)	36 (38%)
Sex						
Male	10 (37%)	12 (46%)	8 (44%)	105 (55%)	108 (57%)	54 (56%)
Female	17 (63%)	14 (54%)	10 (56%)	87 (45%)	83 (43%)	42 (44%)
Han ethnicity	27 (100%)	26 (100%)	18 (100%)	192 (100%)	191 (100%)	96 (100%)
Height, m	1·3 (0·2)	1·3 (0·3)	1·3 (0·3)	1·4 (0·2)	1·4 (0·2)	1·4 (0·2)
Weight, kg	34·3 (15·7)	35·0 (14·9)	34·9 (17·7)	40·4 (19·0)	37·9 (16·9)	39·2 (18·9)

Data are mean (SD) or n (%).

Table 1: **Baseline characteristics**

	1·5 μg group (n=219)	3·0 μg group (n=217)	Aluminium hydroxide only group (n=114)	Total (n=550)	p value*
Solicited adverse reactions within 0–7 days					
Any	51 (23%)	59 (27%)	22 (19%)	132 (24%)	0·28
Grade 1	39 (18%)	51 (24%)	15 (13%)	105 (19%)	0·065
Grade 2	16 (7%)	19 (9%)	9 (8%)	44 (8%)	0·82
Grade 3	2 (1%)	0	0	2 (<1%)	0·36
Injection site adverse reactions					
Pain	36 (16%)	35 (16%)	2 (2%)	73 (13%)	<0·0001
Grade 1	34 (16%)	35 (16%)	2 (2%)	71 (13%)	<0·0001
Grade 2	2 (1%)	0	0	2 (<1%)	0·36
Swelling	3 (1%)	6 (3%)	1 (1%)	10 (2%)	0·50
Grade 1	0	4 (2%)	0	4 (1%)	0·053
Grade 2	3 (1%)	3 (1%)	1 (1%)	7 (1%)	1·0
Induration	0	2 (1%)	0	2 (<1%)	0·20
Grade 1	0	2 (1%)	0	2 (<1%)	0·20
Erythema	0	1 (<1%)	0	1 (<1%)	0·60
Grade 1	0	1 (<1%)	0	1 (<1%)	0·60
Pruritus	3 (1%)	2 (1%)	0	5 (1 %)	0·64
Grade 1	3 (1%)	2 (1%)	0	5 (1%)	0·64
Systematic adverse reactions					
Fever	9 (4%)	11 (5%)	5 (4%)	25 (5%)	0·93
Grade 1	3 (1%)	2 (1%)	2 (2%)	7 (1%)	0·89
Grade 2	4 (2%)	10 (5%)	3 (3%)	17 (3%)	0·22
Grade 3	2 (1%)	0	0	2 (<1%)	0·36
Cough	5 (2%)	8 (4%)	5 (4%)	18 (3%)	0·47
Grade 1	1 (<1%)	4 (2%)	3 (3%)	8 (1%)	0·19
Grade 2	4 (2%)	4 (2%)	2 (2%)	10 (2%)	1·0
Headache	6 (3%)	4 (2%)	3 (3%)	13 (2%)	0·82
Grade 1	3 (1%)	3 (1%)	1 (1%)	7 (1%)	1·0
Grade 2	4 (2%)	1 (<1%)	2 (2%)	7 (1%)	0·39
Anorexia	3 (1%)	4 (2%)	2 (2%)	9 (2%)	0·92
Grade 1	1 (<1%)	3 (1%)	2 (2%)	6 (1%)	0·52
Grade 2	3 (1%)	1 (<1%)	0	4 (1%)	0·54

(Table 2 continues on next page)

	1·5 µg group (n=219)	3·0 µg group (n=217)	Aluminium hydroxide only group (n=114)	Total (n=550)	p value*
(Continued from previous page)					
Diarrhoea	2 (1%)	2 (1%)	4 (4%)	8 (1%)	0·16
Grade 1	2 (1%)	2 (1%)	4 (4%)	8 (1%)	0·16
Nausea	3 (1%)	2 (1%)	2 (2%)	7 (1%)	0·89
Grade 1	3 (1%)	2 (1%)	2 (2%)	7 (1%)	0·89
Mucocutaneous eruption	2 (1%)	2 (1%)	1 (1%)	5 (1%)	1·0
Grade 1	1 (<1%)	1 (<1%)	0	2 (<1%)	1·0
Grade 2	1 (<1%)	1 (<1%)	1 (1%)	3 (1%)	1·0
Vomiting	3 (1%)	1 (<1%)	1 (1%)	5 (1%)	0·85
Grade 1	3 (1%)	1 (<1%)	1 (1%)	5 (1%)	0·85
Muscle pain	4 (2%)	0	0	4 (1%)	0·078
Grade 1	2 (1%)	0	0	2 (<1%)	0·36
Grade 2	2 (1%)	0	0	2 (<1%)	0·36
Fatigue	1 (<1%)	1 (<1%)	1 (1%)	3 (1%)	1·0
Grade 1	1 (<1%)	1 (<1%)	1 (1%)	3 (1%)	1·0
Grade 2	1 (<1%)	0	0	1 (<1%)	1·0
Hypersensitivity	0	0	1 (1%)	1 (<1%)	0·21
Grade 1	0	0	1 (1%)	1 (<1%)	0·21
Unsolicited adverse reactions within 0–28 days					
Any	11 (5%)	15 (7%)	9 (8%)	35 (6%)	0·52
Grade 1	2 (1%)	3 (1%)	3 (3%)	8 (1%)	0·43
Grade 2	10 (5%)	12 (6%)	7 (6%)	29 (5%)	0·75
Overall adverse reactions within 0–28 days					
Any	56 (26%)	63 (29%)	27 (24%)	146 (27%)	0·55
Grade 1	40 (18%)	52 (24%)	18 (16%)	110 (20%)	0·16
Grade 2	22 (10%)	24 (11%)	15 (13%)	61 (11%)	0·67
Grade 3	2 (1%)	0	0	2 (<1%)	0·36

Data are n (%), representing the total number of participants who had adverse reactions (ie, adverse events related to vaccination). Results are broken down by dose and age group in the appendix (pp 2–10). *For differences across all groups.

Table 2: **Adverse reactions reported within 28 days after the first and the second dose of vaccine or alum only in phase 1 and phase 2**

552 enrolled participants received the first dose of vaccine or alum only (71 in phase 1 and 479 in phase 2) and were included in the safety population (figure 1). 69 (96%) participants in phase 1 received the second dose and all were eligible for the immunogenic evaluation at day 28 after the second dose (per-protocol population; figure 1). In phase 2, 467 (97%) participants received the second dose and 460 (96%) were included in the per-protocol population (figure 1). Seven participants were excluded because one received tetanus immunoglobulin at day 14 after the second dose, five did not have a blood sample taken at 28 days after the second dose, and one took a blood sample outside of the specified time window. The demographic characteristics of the participants were similar in terms of sex, mean age, height, weight, and ethnicity among groups. The mean age of study participants was 8·3 years (SD 4·0) in phase 1, including 24 (34%) of 71 participants aged 3–5 years, 24 (34%) aged 6–11 years, and 23 (32%) aged 12–17 years. The mean age of study participants was 9·2 years (3·9) in phase 2, including 119 (25%) of 479 participants aged 3–5 years, 180 (38%) aged 6–11 years, and 180 (38%) aged 12–17 years (table 1).

The safety data of the phase 1 and phase 2 trial were combined for analysis because the same batches of the vaccine and alum only and the same safety observation method were used. 146 (27%) of 550 participants reported at least one adverse reaction within 28 days of either vaccination, and the proportions of participants with any adverse reactions were similar across groups. Most adverse reactions were mild (grade 1) and moderate (grade 2) in severity. Only two (<1%) of 550 had grade 3 adverse reactions. Most adverse reactions occurred within 7 days after vaccination and participants recovered within 48 h. The most common reactions were injection site pain (73 [13%] participants) and fever (25 [5%]). Except for a higher prevalence of injection site pain in two vaccine groups than that in alum-only group, there

	1·5 µg group		3·0 µg group		Aluminium hydroxide only group		p value	
	Rate	% (95%) CI	Rate	% (95%) CI	Rate	% (95%) CI	Three groups	1·5-µg *vs* 3·0-µg group
Phase 1								
Total	27/27	100·0% (87·2–100·0)	26/26	100·0% (86·8–100·0)	0/16	0·0% (0·0–20·6)	<0·0001	1·0
3–5 years	9/9	100·0% (66·4–100·0)	9/9	100·0% (66·4–100·0)	0/5	0·0% (0·0–52·2)	<0·0001	1·0
6–11 years	9/9	100·0% (66·4–100·0)	9/9	100·0% (66·4–100·0)	0/6	0·0% (0·0–45·9)	<0·0001	1·0
12–17 years	9/9	100·0% (66·4–100·0)	8/8	100·0% (63·1–100·0)	0/5	0·0% (0·0–52·2)	<0·0001	1·0
Phase 2								
Total	180/186	96·8% (93·1–98·8)	180/180	100·0% (98·0–100·0)	0/94	0·0% (0·0–3·9)	<0·0001	0·030
3–5 years	46/46	100·0% (92·3–100·0)	45/45	100·0% (92·1–100·0)	0/24	0·0% (0·0–14·2)	<0·0001	1·0
6–11 years	68/69	98·6% (92·2–100·0)	68/68	100·0% (94·7–100·0)	0/35	0·0% (0·0–10·0)	<0·0001	1·0
12–17 years	66/71	93·0% (84·3–97·7)	67/67	100·0% (94·6–100·0)	0/35	0·0% (0·0–10·0)	<0·0001	0·059

Data are n/N (% [95% CI]).

Table 3: **Seroconversion rates of neutralising antibody responses to live SARS-CoV-2 28 days after the second dose**

were no significant differences in the prevalence of other solicited or unsolicited reactions among the three groups (table 2). In an exploratory analysis by age, the prevalence of adverse reactions was highest in participants aged 12–17 years (72 [35%] of 203 participants) followed by 3–5 years (37 [26%] of 143 participants) and 6–11 years (37 [18%] of 204 participants; appendix pp 8–10). As of June 12, 2021, only one participant in the alum-only group has reported one serious adverse event (pneumonia; appendix p 15), which was considered to be unrelated to vaccination. Additionally, only two (3%) of 71 participants at day 3 after the first dose and two (3%) of 69 participants after the second dose in phase 1 had a significant increase of laboratory indicator (appendix p 11).

In phase 1, none of the participants had any detectable neutralising antibody response against live SARS-CoV-2 at baseline (appendix p 12). The seroconversion rates at day 28 after the second dose were 27 (100%) of 27 participants in the 1·5 µg group (GMT 55·0 [95% CI 38·9–77·9]) and 26 (100%) of 26 in the 3·0 µg group (117·4 [87·8–157·0]). The GMT of the 3·0 µg group was significantly higher than that of the 1·5 µg group (p=0·0012; table 3, figure 2, appendix p 12). Testing for neutralising antibodies in all alum-only recipients was negative after vaccination (appendix p 12). In an exploratory analysis by age, seroconversion rates at day 28 after the second dose of 1·5 µg or 3·0 µg vaccine were all 100% in participants aged 3–5 years, 6–11 years, and 12–17 years, with the GMTs ranging from 45·9 to 212·6 (figure 2, appendix p 14).

In phase 2, none of the participants had any detectable neutralising antibody response at baseline (appendix p 13). After the second dose of vaccination, the seroconversion rates were 180 (95% CI 96·8% [93·1–98·8]) of 186 participants in the 1·5 µg group (GMT 86·4 [73·9–101·0]) and 180 (100·0% [98·0–100·0]) of 180 participants in the 3·0 µg group (142·2 [124·7–162·1]). The seroconversion rate and GMT of the 3·0 µg group were higher than those of the 1·5 µg group (p=0·030 and p<0·0001; table 3, figure 2, appendix p 13). Neutralising antibodies in all alum-only recipients were negative after vaccination (appendix p 13). In an exploratory analysis by age, the seroconversion rates at day 28 after the second dose were higher than 93% in the 1·5 µg and 3·0 µg groups for participants aged 3–5 years, 6–11 years, and 12–17 years, with the GMTs ranging from 78·3 to 146·0 (figure 2, appendix p 14).

Discussion

To our knowledge, this is the first report of immunogenicity and safety of COVID-19 candidate vaccine among children as low as 3 years old. We found that two

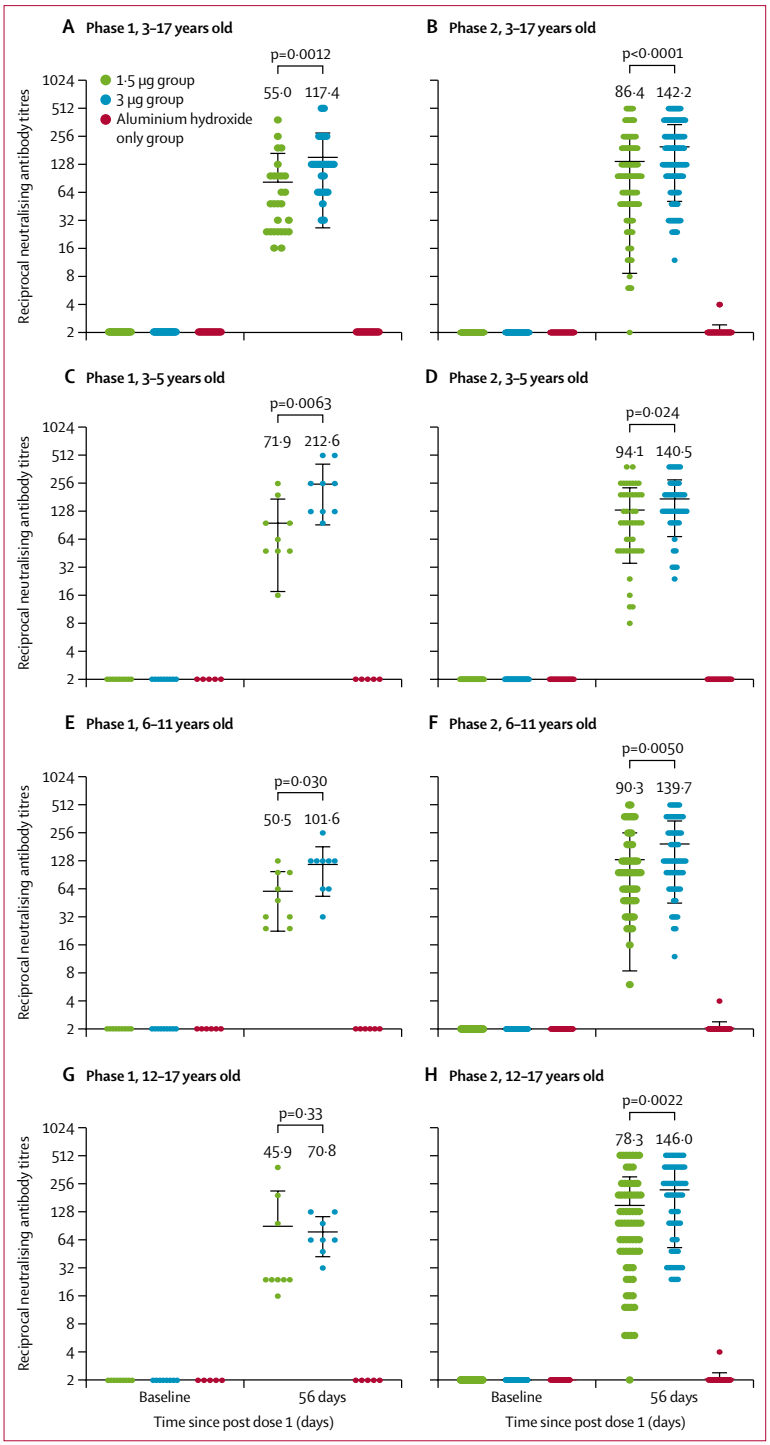


Figure 2: **Antibody titres of neutralising antibodies to live SARS-CoV-2 induced after two doses of CoronaVac or aluminium hydroxide diluent only in phase 1 and phase 2 trials**

GMT=geometric mean titre.The error bars indicate the 95% CI of the GMT and the spots indicate the individual antibody titres, with the number above the spots showing the GMT estimate. Only p values between 1·5 µg and 3·0 µg groups after the second vaccination are shown in the figure. All p values for all data are in the appendix (pp 12–13)

doses of the CoronaVac were safe and well tolerated at doses of 1·5 µg and 3·0 µg among children and adolescents aged 3–17 years old. The prevalence of adverse reactions in different dose groups was similar, indicating that there was no dose-related concern on safety. Most reactions were mild to moderate in severity and transient. Injection-site pain was the most reported symptom. The results were similar to our study of adults and elderly.[17,18] Furthermore, the higher grade 1 injection site pain reported by adolescents aged 12–17 years was the main reason for the higher prevalence of adverse reactions in this population compared with children aged 3–5 years and 6–11 years. None of the serious adverse events reported during the trial was related to vaccination.

CoronaVac was immunogenic in children and adolescents aged 3–17 years. The seroconversion rates of neutralising antibody in children and adolescents with both doses were over 96% after the two-dose vaccination. The GMTs of 142·2 in the 3·0 µg groups were higher than that of 86·4 in the 1·5 µg group in phase 2; however, even the GMT of 86·4 induced better immunogenicity compared with adults aged 18–59 years (44·1) and those aged 60 years and older (42·2) who received a 3·0 µg dose of vaccine with the same immunisation schedule.[17,18] Age plays an important role in antibody response to vaccine.[27] Decreasing responses to vaccination with increasing age have been shown in other vaccines, such as hepatitis B vaccine, seasonal influenza, pneumococcal disease, tetanus, pertussis, and diphtheria.[27,28] The results implied that a lower dose of vaccine could induce higher immune response in children and adolescents.

In an exploratory analysis stratified by age, we did not observe significant differences in neutralising antibody responses between age groups (3–5 years, 6–11 years, and 12–17 years) after the second vaccination (appendix p 14). GMTs in phase 1 decreased with age in recipients of the same vaccine, whereas they were similar in phase 2. Small sample size might account for the change trends of GMT in phase 1. In each age group, there were significant differences in GMTs between the 1·5 µg and 3·0 µg groups after the second dose, except in the group aged 12–17 years old in phase 1. Taken together, the 3·0 µg dose of CoronaVac induced higher immune responses in all age groups compared with the 1·5 µg dose.

Evidence from various studies supports the important role of T-cell responses to SARS-CoV-2 infection,[29] and such responses have been found with use of different vaccine platforms, including mRNA, viral vectors, and recombinant proteins.[30] In this study, T cell responses were not assessed, which was a limitation of the study design. However, a study in Chile found a significant induction of a T-cell response characterised by the secretion of interferon-gamma following vaccination of CoronaVac in a population aged 18 years and older,[19], which was different from the lower response observed in our phase 1 trial among adults aged 18–59 years.[17] Another inactivated SARS-CoV-2 vaccine, BBV152, has also been reported to induced a Th1-biased response.[21,24] Future studies are needed to assess the responses of type 1 and type 2 T-helper cells by inactivated vaccines.

This study has some further limitations. First, the sample size of this study is relatively small per age group and all study populations were of Han ethnicity. Further studies will be done in different regions and multiethnic populations to collect more data to provide scientific evidence for immune strategy. Second, at the time of the report, long-term immunogenicity and safety could not be available, although the participants will be followed up for at least 1 year. Finally, the calculated p values cannot support any powerful statistical conclusions in this study, which are only for reference and should be interpreted with caution.

In conclusion, CoronaVac was well tolerated and safe, and induced humoral responses in children and adolescents aged 3–17 years. Among the two doses evaluated, the neutralising antibody titres induced by a 3·0 µg dose were higher than those of the 1·5 µg dose. The results support the use of 3·0 µg dose with a two-immunisation schedule for further studies in children and adolescents.

Contributors

QL, QG, YZ, BH, and YS designed the trial and study protocol. BH, WY, and ML contributed to the literature search. All authors had access to data, and YS and QL verified the data. BH and WY wrote the first draft manuscript. QG, QL, YS, ML, XL, and YZ contributed to the data interpretation and revision of the manuscript. ZJ and QS contributed to data analysis. LW monitored the trial. QM and WJ were responsible for the site work including the recruitment, follow-up, and data collection, and ZW was the site coordinator. CL were responsible for the laboratory analysis. All the authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

QG and XL are employees of Sinovac Life Sciences. YS, WY, and LW are employees of Sinovac Biotech. All other authors declare no competing interests.

Data sharing

The individual participant-level data that underlie the results reported in this Article will be shared after de-identification (text, tables, figures, and appendices). This clinical trial is ongoing, and all the individual participant data will not be available until the immune persistence evaluation is completed. The data will be available immediately after publication and finalisation of the completed clinical study report for at least 6 months. Supporting clinical documents including the study protocol and statistical analysis plan and the informed consent form will be available immediately following publication of this Article for at least 1 year. Information on how to access the supporting clinical documents is available online. Researchers who provide a scientifically sound proposal will be allowed to access to the de-identified individual participant data. Proposals should be sent to the corresponding author. These proposals will be reviewed and approved by the sponsor, investigators, and collaborators on the basis of scientific merit. To gain access, data requestors will need to sign a data access agreement.

Acknowledgments

This study was funded by the National Key Research and Development Program and Beijing Science and Technology Program.

References

1 WHO. WHO coronavirus disease (COVID-19) dashboard. 2021. https://covid19whoint/ (accessed June 12, 2021).

2 Zimmermann P, Curtis N. Coronavirus infections in children including COVID-19: An overview of the epidemiology, clinical features, diagnosis, treatment and prevention options in children. *Pediatr Infect Dis J* 2020; **39:** 355–68.
3 European Centre for Disease Prevention and Control. Paediatric inflammatory multisystem syndrome and SARS-CoV-2 infection in children. May 15, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-risk-assessment-paediatric-inflammatory-multisystem-syndrome-15-May-2020.pdf (accessed March 25, 2021).
4 Maltezou HC, Magaziotou I, Dedoukou X, et al. Children and adolescents with SARS-CoV-2 Infection: epidemiology, clinical course and viral loads. *Pediatr Infect Dis J* 2020; **39:** e388–92.
5 Snape MD, Viner RM. COVID-19 in children and young people. *Science* 2020; **370:** 286–88.
6 Kamidani S, Rostad CA, Anderson EJ. COVID-19 vaccine development: a pediatric perspective. *Curr Opin Pediatr* 2021; **33:** 144–51.
7 Ebina-Shibuya R, Namkoong H, Shibuya Y, Horita N. Multisystem inflammatory syndrome in children (MIS-C) with COVID-19: insights from simultaneous familial Kawasaki disease cases. *Int J Infect Dis* 2020; **97:** 371–73.
8 Kao CM, Orenstein WA, Anderson EJ. The importance of advancing SARS-CoV-2 vaccines in children. *Clin Infect Dis* 2020; ciaa712.
9 Yang HS, Costa V, Racine-Brzostek SE, et al. Association of age with SARS-CoV-2 antibody response. *JAMA Netw Open* 2021; **4:** e214302.
10 WHO. Draft landscape of COVID-19 candidate vaccines. 2021. https://wwwwhoint/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines (accessed June 12, 2021).
11 Creech CB, Walker SC, Samuels RJ. SARS-CoV-2 vaccines. *JAMA* 2021; **325:** 1318–20.
12 Palacios R, Batista AP, Albuquerque CSN, et al. Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in Brazil: the PROFISCOV study. *SSRN* 2021; published online April 14, 2021. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3822780 (preprint).
13 Miller NS. COVID-19 vaccines in children: research to guide your news coverage. 2021. https://journalistsresourceorg/health/covid-19-vaccine-in-children/ (accessed April 27, 2021).
14 National Institutes of Health. Safety and immunogenicity study of inactivated vaccine for prevention of COVID-19. 2020. https://clinicaltrialsgov/ct2/show/NCT04551547?cond=NCT04551547&draw=2&rank=1 (accessed April 27, 2021).
15 Pfizer. Pfizer-Biontech announce positive topline results of pivotal COVID-19 vaccine study in adolescents. March 31, 2021. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-biontech-announce-positive-topline-results-pivotal (accessed April 27, 2021).
16 Gao Q, Bao L, Mao H, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. *Science* 2020; **369:** 77–81.
17 Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21:** 181–92.
18 Wu Z, Hu Y, Xu M, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21:** 803–12.
19 Bueno SM, Abarca K, González PA, et al. Interim report: safety and immunogenicity of an inactivated vaccine against SARS-CoV-2 in healthy Chilean adults in a phase 3 clinical trial. *medRxiv* 2021; published online April 1. https://doi.org/10.1101/2021.03.31.21254494 (preprint).
20 Che Y, Liu X, Pu Y, et al. Randomized, double-blinded and placebo-controlled phase II trial of an inactivated SARS-CoV-2 vaccine in healthy adults. *Clin Infect Dis* 2020; ciaa1703. https://doi.org/10.1093/cid/ciaa1703.
21 Ella R, Vadrevu KM, Jogdand H, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: a double-blind, randomised, phase 1 trial. *Lancet Infect Dis* 2021; **21:** 637–46.
22 Xia S, Duan K, Zhang Y, et al. Effect of an inactivated vaccine against SARS-CoV-2 on safety and immunogenicity outcomes: interim analysis of 2 randomized clinical trials. *JAMA* 2020; **324:** 951–60.
23 Xia S, Zhang Y, Wang Y, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. *Lancet Infect Dis* 2021; **21:** 39–51.
24 Ella R, Reddy S, Jogdand H, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: interim results from a double-blind, randomised, multicentre, phase 2 trial, and 3-month follow-up of a double-blind, randomised phase 1 trial. *Lancet Infect Dis* 2021; published online March 8. https://doi.org/10.1016/S1473-3099(21)00070-0.
25 Chine National Medical Products Administration. Guidelines for grading standards of adverse events in clinical trials of preventive vaccines. https://www.nmpa.gov.cn/xxgk/ggtg/qtggtg/20191231111901460html (accessed March 29, 2020).
26 Li YP, Liang ZL, Gao Q, et al. Safety and immunogenicity of a novel human enterovirus 71 (EV71) vaccine: a randomized, placebo-controlled, double-blind, phase I clinical trial. *Vaccine* 2012; **30:** 3295–303.
27 Kang G, Chen H, Ma F, et al. Comparison of the effect of increased hepatitis B vaccine dosage on immunogenicity in healthy children and adults. *Hum Vaccin Immunother* 2016; **12:** 2312–16.
28 Van Der Meeren O, Crasta P, Cheuvart B, De Ridder M. Characterization of an age-response relationship to GSK's recombinant hepatitis B vaccine in healthy adults: an integrated analysis. *Hum Vaccin Immunother* 2015; **11:** 1726–29.
29 Sauer K, Harris T. An effective COVID-19 vaccine needs to engage T cells. *Front Immunol* 2020; **11:** 581807.
30 Rha MS, Kim AR, Shin EC. SARS-CoV-2-Specific T cell responses in patients with COVID-19 and unexposed individuals. *Immune Netw* 2021; **21:** e2.

7. Dose de reforço multiplica anticorpos

7.1. Reforço da CoronaVac protege em 98% contra casos graves e mortes por Covid-19, afirma estudo de Hong Kong

Um estudo realizado em Hong Kong durante o surto da variante ômicron do SARS-CoV-2, no primeiro trimestre de 2022, mostrou que a administração de três doses da CoronaVac forneceu proteção de 98% contra casos graves e mortes por Covid-19, principalmente entre idosos. O trabalho foi revisado por pares e publicado na revista The Lancet Infectious Diseases e conduzido por pesquisadores da Escola de Saúde Pública e da Faculdade de Medicina da Universidade de Hong Kong. Os resultados foram publicados anteriormente em formato preprint.

Até dezembro de 2021, Hong Kong se destacou no controle da pandemia devido ao isolamento social restritivo imposto pelo governo. Mas, no início de janeiro de 2022, explodiu na cidade o surto da sublinhagem BA.2 da ômicron, que acabou resultando em 741 mil casos de Covid-19 e 8.875 casos graves ou mortes.

Até meados de abril de 2022, 61% da população com idade entre 40 e 59 anos haviam tomado três doses da vacina. Esse percentual foi de 39% na faixa entre 70 e 79 anos e de 15% entre os idosos com 80 anos ou mais.

A administração de duas doses da CoronaVac mostrou uma eficácia de 91,7% para evitar casos severos de Covid-19 em adultos entre 20 e 59 anos. Já entre os idosos com 60 a 69 anos a eficácia de duas doses chegou a 79,3%, e 74,3% entre 70 e 79 anos.

No caso da dose de reforço, os resultados foram ainda melhores. "Estimamos que três doses ofereceram proteção muito alta contra doença grave (97,9%) e óbito (98,6%) em todas as faixas etárias", dizem os autores do estudo.

Especificamente entre os idosos, três doses da CoronaVac mostraram uma eficiência para evitar casos graves e mortes de 97,4%, 95,4% e 97,3%, nas faixas dos 60 aos 69 anos, dos 70 aos 79 anos, e de 80 anos ou mais, respectivamente.

Publicado em: 15/7/2022

Vaccine effectiveness of one, two, and three doses of BNT162b2 and CoronaVac against COVID-19 in Hong Kong: a population-based observational study

Martina E McMenamin, Joshua Nealon, Yun Lin, Jessica Y Wong, Justin K Cheung, Eric H Y Lau, Peng Wu, Gabriel M Leung, Benjamin J Cowling

Summary

Background **Hong Kong maintained low circulation of SARS-CoV-2 until a major community epidemic of the omicron (B.1.1.529) sublineage BA.2 began in January, 2022. Both mRNA (BNT162b2 [Fosun Pharma-BioNTech]) and inactivated CoronaVac (Sinovac, Beijing, China) vaccines are widely available; however, vaccination coverage has been low, particularly in older adults aged 70 years or older. We aimed to assess vaccine effectiveness in this predominantly infection-naive population.**

Methods **In this observational study, we used individual-level case data on mild or moderate, severe or fatal, and fatal disease in patients hospitalised with COVID-19 along with census information and coverage data of BNT162b2 and CoronaVac. We used a negative binomial model, adjusting for age, sex, and calendar day to estimate vaccine effectiveness of one, two, and three doses of both BNT162b2 and CoronaVac vaccines, and relative effectiveness by number of doses and vaccine type.**

Findings **Between Dec 31, 2020, and March 16, 2022, 13·2 million vaccine doses were administered in Hong Kong's 7·4-million population. We analysed data from confirmed cases with mild or moderate (n=5566), severe or fatal (n=8875), and fatal (n=6866) COVID-19. Two doses of either vaccine protected against severe disease and death within 28 days of a positive test, with higher effectiveness among adults aged 60 years or older with BNT162b2 (vaccine effectiveness 89·3% [95% CI 86·6–91·6]) compared with CoronaVac (69·9% [64·4–74·6]). Three doses of either vaccine offered very high levels of protection against severe or fatal outcomes (97·9% [97·3–98·4]).**

Interpretation **Third doses of either BNT162b2 or CoronaVac provide substantial additional protection against severe COVID-19 and should be prioritised, particularly in older adults older than 60 years and others in high-risk populations who received CoronaVac primary schedules. Longer follow-up is needed to assess duration of protection across different vaccine platforms and schedules.**

Funding **COVID-19 Vaccines Evaluation Program, Chinese Center for Disease Control and Prevention.**

Lancet Infect Dis 2022; 22: 1435–43

Published Online July 15, 2022 https://doi.org/10.1016/S1473-3099(22)00345-0

This online publication has been corrected. The corrected version first appeared at thelancet.com/infection on July 28, 2022

WHO Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China (M E McMenamin PhD, J Nealon PhD, Y Lin MPH, J Y Wong PhD, J K Cheung BSc, E H Y Lau PhD, P Wu PhD, Prof G M Leung MD, Prof B J Cowling PhD); **Laboratory of Data Discovery for Health, Hong Kong Science and Technology Park, Hong Kong Special Administrative Region, China** (E H Y Lau, P Wu, Prof G M Leung, Prof B J Cowling)

Correspondence to:
Dr Joshua Nealon, WHO Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China
jnealon@hku.hk

or

Prof Benjamin J Cowling, WHO Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China
bcowling@hku.hk

Introduction

Hong Kong (population 7·4 million) has pursued a COVID-19 elimination strategy since January, 2020, involving stringent physical distancing measures, border entry restrictions, isolation of cases, quarantine of close contacts, and the use of personal protective measures.[1] Consequently, the disease had been largely controlled until December, 2021, with four previous epidemic waves resulting in 12 631 cases (<2 per 1000) and 213 deaths (<3 per 100 000). Since February, 2021, both inactivated (CoronaVac [Sinovac, Beijing, China]) and mRNA (BNT162b2 [Fosun Pharma-BioNTech]) vaccines have been widely available with residents older than 5 years offered the choice of either. However, by January, 2022, two-dose vaccine coverage had only reached 46% in adults aged 70–79 years of age and 18% in those aged 80 years and older.

A major community epidemic of the SARS-CoV-2 omicron (B.1.1.529) variant sublineage BA.2 began in early January, 2022, resulting in 741 708 laboratory confirmed cases, 441 945 cases positive by rapid antigen tests, and 8856 deaths until April 15, 2022.[2] Vaccination coverage has since increased but remains low in older people, with two-dose coverage at 62% in those aged 80 years and older by June 27, 2022. Third vaccine doses were recommended first for priority groups and then for members of the general public older than 18 years on Jan 1, 2022, to be given 6 months after the second dose.[3] As of April 18, 2022, third dose uptake has been highest in those aged 40–59 years (61%) and lower in older adults (39% in those aged 70–79 years and 15% in those aged ≥80 years). Efforts to increase uptake are underway, including reducing the duration between first and second doses for care-home residents; extending vaccination clinic operating hours; and deploying vaccine outreach teams to care homes, housing estates, and residents with reduced mobility.[4]

International data have shown that vaccination with BNT162b2 reduces the frequency of severe outcomes and, to a lesser extent, infection for variants circulating

Research in context

Evidence before this study

A systematic review by Higdon and colleagues identified 22 efficacy studies for 15 COVID-19 vaccine candidates and 107 observational studies describing performance of eight COVID-19 vaccines. Their review included 86 studies of the vaccine effectiveness of BNT162b2 (Fosun Pharma-BioNTech) and six studies of CoronaVac (Sinovac, Beijing, China) effectiveness. Four BNT162b2 studies and none of the CoronaVac studies were done in areas with circulation of the omicron (B.1.1.529) variant. We searched medRxiv, PubMed, and SSRN using the following search terms: "((vaccine effectiveness) AND (omicron) AND (BA.2)) AND ((BNT162b2) OR (Comirnaty) OR (Coronavac))", restricting the search from Nov 24, 2021, to March 16, 2022, to coincide with when the omicron variant was reported to WHO and the cutoff for inclusion in our study. We found no published articles and 32 preprints, five of which estimated vaccine effectiveness using clinical outcome data. Of these, only two studies estimated mRNA vaccine effectiveness against the BA.2 sublineage (both in Qatar). The authors reported vaccine effectiveness estimates of BNT162b2 against COVID-19 hospitalisation and death in the range of 70–80% any time after the second dose, and greater than 90% after the third dose. No estimates of the CoronaVac vaccine against BA.2 have been reported to date. Because of previously low SARS-CoV-2 circulation, no previous estimates of COVID-19 vaccine effectiveness in Hong Kong have been published. Given that both CoronaVac and BNT162b2 are widely in use, the BA.2 sublineage is in circulation, and population immunity is almost entirely vaccine-derived, Hong Kong represents a unique environment for monitoring vaccine effectiveness, and vaccine performance might be expected to vary from that of other settings.

Added value of this study

To our knowledge, we present the first assessment of the vaccine effectiveness of mRNA and inactivated vaccines, and relative effectiveness of three versus two doses, against the omicron BA.2 sublineage, in an immunologically-naive population. Recipients of at least two doses of BNT162b2 vaccine had at least 85% vaccine effectiveness and three doses of either BNT162b2 or CoronaVac had greater than 95% vaccine effectiveness against severe or fatal outcomes, irrespective of age. Greater protection was observed among those who received two doses of BNT162b2 compared with two doses of CoronaVac across all age groups. Third vaccine doses were associated with a relative effectiveness versus two doses of 68–97% against severe and fatal outcomes, with the caveat that third doses were recently administered (within a median of 44–61 days), and the vaccine effectiveness might wane. These findings are the first estimates of vaccine effectiveness from Hong Kong and will therefore provide important contributions to vaccination policy in areas where two-dose and three-dose vaccine coverage in older adults remains low.

Implications of all the available evidence

Our results show the importance and urgency of achieving high vaccination coverage in a population that has acquired minimal protection from natural infection, particularly in those most at risk, with a preference for BNT162b2 in a two-dose schedule. Older adults (>60 years) and those in high-risk groups who have received two doses of an inactivated vaccine are strongly recommended to receive a third dose to obtain high levels of protection. A third dose of either an inactivated or mRNA vaccine provides high protection from severe and fatal COVID-19, and innovative public health policies to improve coverage in older adults should be urgently followed to minimise avoidable COVID-19 morbidity and mortality. Additional, longer-term research is needed to understand the duration of protection associated with different vaccines, including heterologous schedules.

before omicron.[5–8] Waning protection has been observed in multiple contexts, in particular against infection,[9–11] and studies have provided early indications of reduced effectiveness of BNT162b2 against omicron.[12,13] Evidence on vaccine performance against the more transmissible omicron sublineage BA.2 remains scarce, as are data on the performance of the inactivated CoronaVac vaccine against previously circulating variants. Some observational evidence suggests strong and durable protection against severe disease and death from both vaccines, with transient protection against milder symptomatic disease.[14–17] With a largely infection-naive population and two COVID-19 vaccines in widespread use, Hong Kong represents a unique environment for monitoring vaccine effectiveness against omicron lineage BA.2. We aimed to estimate vaccine effectiveness of one, two, and three doses of BNT162b2 and CoronaVac, their relative effectiveness, and the additional protection offered by third doses against mild and moderate infections, severe disease, and death.

Methods

Study design and population

In this observational study, we assessed vaccine effectiveness of the BNT162b2 and CoronaVac vaccines using an ecological study design, previously used in Israel.[18] The study population was Hong Kong residents aged 20 years and older. The population vaccinated with zero, one, two or three doses of either vaccine at risk at a given time was derived using vaccination programme and census data. Information on all laboratory-confirmed SARS-CoV-2 infections was obtained from individual-level surveillance data provided by the Hong Kong Centre for Health Protection and linked to clinical outcome data provided by the Hospital Authority of Hong Kong.

This project received approval from the Institutional Review Board of the University of Hong Kong (UW 20-341). Informed consent was not required.

Procedures

Extensive PCR testing for SARS-CoV-2 is done in public hospitals, community test centres, and private laboratories in Hong Kong. Testing is free-of-charge or low cost and required for those with COVID-19-like symptoms or following contact tracing based on exposure history or residential location. Regular screening is also required for those in certain professions, in particular those working with older adults or vulnerable people. Positive rapid test results have been recognised as confirmed infections since Feb 25, 2022. Data on all confirmed cases between Dec 31, 2021, and March 16, 2022, were extracted and cases that were classified as imported—ie, detected in on-arrival quarantine—were excluded because of their non-representative histories of SARS-CoV-2 exposure and vaccination. Individuals with missing age or sex information were excluded, as well as vaccinated individuals with missing information on vaccine type or date of any vaccine dose. Sequencing of a subset of cases each day indicated that less than 1% of cases and deaths during the fifth wave occurred with the variant B.1.617.2 (delta), with the remaining infections attributed to omicron sublineage BA.2 (Poon L, University of Hong Kong, personal communication).[19]

Until mid-February, 2022, all patients with SARS-CoV-2 infections were admitted to hospitals regardless of symptoms. After this point hospitalisation was reserved for patients with more severe disease, and patients with milder disease were required to isolate at Government quarantine facilities or at home. Electronic medical records from patients attending hospitals managed by the Hospital Authority of Hong Kong are stored in the centralised clinical data analysis and reporting system, including information on demographics, laboratory results, and clinical data.[20] We extracted records of all hospitalisations with confirmed COVID-19 between Dec 31, 2021, and March 16, 2022, from data provided on April 14, 2022, to capture all deaths within 28 days of laboratory confirmation, including those with mild or moderate disease before Feb 16, 2022, and severe disease or death at any time. Records were regularly updated and the worst condition during hospitalisation was documented as either mild (non-fatal, non-serious, and non-critical), serious (oxygen supplement of 33 litres per min), or critical (admitted to an intensive care unit [ICU], intubated, requiring extracorporeal membrane oxygenation [ECMO], or in shock). Deaths within 28 days of a positive SARS-CoV-2 test were considered COVID-19 fatalities. We defined severe disease as any serious or critical condition and combined this definition with COVID-19 fatality to form the severe or fatal outcome (appendix p 1). This categorisation aimed to minimise misclassification bias arising from coding anomalies whereby oxygen supplementation or other clinical information requiring manual data entry might have been omitted from patient records, and to include individuals who died from COVID-19-related causes before meeting the criteria for serious or critical episodes due to health-care capacity becoming overwhelmed.

Data on the estimated population size at the end of 2021 by age (years) were obtained from the Census and Statistics Department of the Hong Kong Government. Data on the number of people vaccinated with BNT162b2 or CoronaVac vaccines each day since Feb 22, 2021, are available in a vaccination database provided by the Department for Health. We extracted data on all vaccinations that had occurred up to March 16, 2022, by age, sex, and the type and date of receipt of each vaccine dose on April 12, 2022 (appendix pp 2–3). Individuals with laboratory-confirmed SARS-CoV-2 infection who received vaccines other than BNT162b2 or CoronaVac, a mixed primary series (one dose of BNT162b2 and one dose of CoronaVac), or a third dose that was different from the primary series were excluded from the analysis. Individuals with known previous SARS-CoV-2 infection were also excluded.

For the **Department of Health Dashboard** see https://www.covidvaccine.gov.hk/en/dashboard

Statistical analysis

We estimated incidence rate ratios (IRRs) according to the number of vaccine doses received (none, one, two, or three) for each of the mild or moderate, severe or fatal, and fatal COVID-19 outcomes. Data were stratified by age group (20–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, 70–79 years, ≥80 years), sex, vaccine type, and calendar day throughout the study period. Vaccination status was categorised according to the date of vaccination plus a 14-day lag for all doses, to allow for the delay in immune response to vaccination. Daily numbers of people in each vaccination category were inferred from the uptake data assuming that individuals received the same vaccine for first and second dose (aligned with Hong Kong guidelines), and using aggregate data by age on vaccine switching for the third dose. The population at risk in each stratum was matched to the report date of cases, and individuals with previous SARS-CoV-2 infection within each group were removed from the population at risk at each timepoint. This process was repeated for each outcome of interest. IRRs were estimated in adults younger than 60 years and in those aged 60–69 years, 70–79 years, and 80 years or older for all outcomes, using negative binomial regression models for the daily counts of cases, adjusting for age group, sex, and calendar day and including the logarithm of person-time as an offset term in the model to account for differing numbers at risk within each strata. Each stratified daily case count was considered as a single observation, resulting in a total of 7448 observations across all age groups. Vaccine effectiveness was defined as (1–IRR)×100%. We performed sensitivity analyses calculating incidence per calendar

See Online for appendix

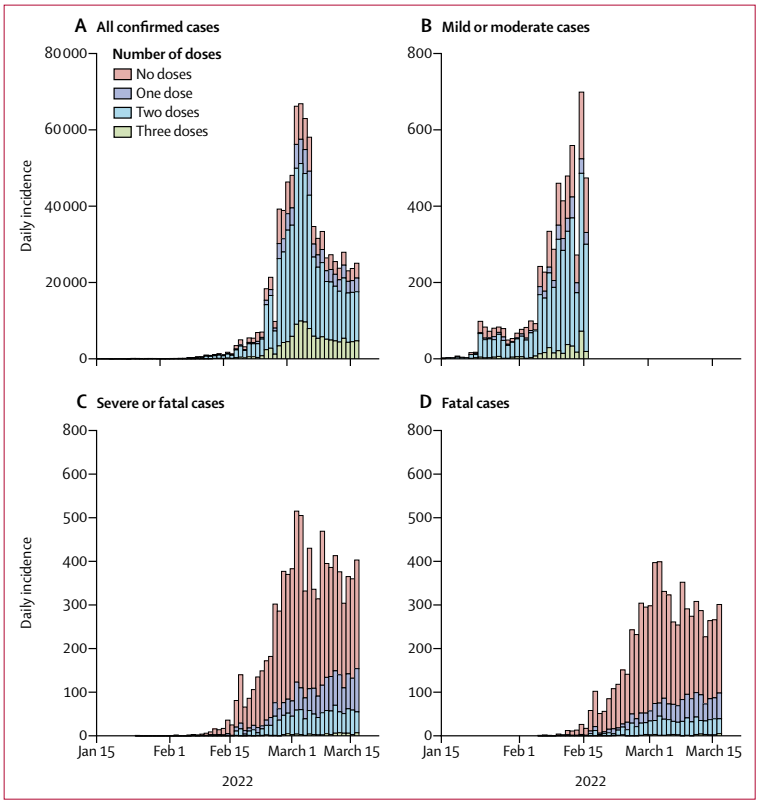


Figure 1: **Daily incidence of cases and deaths by vaccination status**
(A) All confirmed COVID-19 cases. (B) Mild or moderate cases in the early part of the fifth wave before Feb 15, 2022. (C) Severe or fatal cases. (D) Deaths throughout the fifth wave in Hong Kong. Severe disease was defined as having ever been listed as serious or critical during hospitalisation for COVID-19 or having a fatal outcome within 28 days of positive test. Vaccination status was categorised according to the number of doses received plus a 14-day lag for all doses, to allow for the immune response to vaccination. Mild cases were only included up until Feb 15, 2022, to account for change in admission criteria.

week and assuming a 7-day lag instead of 14 days for immune response to vaccination. All analyses were done with R (version 4.1.1).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Dec 31, 2021, and March 16, 2022, 962 557 people had confirmed SARS-CoV-2 infection. Of these, 5566 (0·6%) people were recorded as having mild or moderate disease between Dec 31, 2021, and Feb 15, 2022, and were included in the analysis, after excluding an additional 37790 (3·9%) mild cases occurring between Feb 16 and March 16, 2022, due to changes in admission criteria. 40 (<0·1%) cases were listed as mild but with fatal outcomes; these individuals were included in the severe or fatal outcomes group. During the entire study period severe or fatal disease occurred in 8875 (0·9%) people and 6866 (0·7%) deaths occurred in 462 638 762 person-days (figure 1; appendix p 4). 30 reinfected cases were excluded, along with two individuals with unknown age and nine individuals with differing numbers of doses administered according to different datasets and who we therefore considered of unknown vaccination status.

Up to March 16, 2022, 13·2 million vaccine doses had been administered. Severe disease or death occurred a median of 167 days (IQR 76–209) days after the second vaccination in those vaccinated with two doses of BNT162b2, and 125 days (47–166) among those who received two doses of CoronaVac (table 1). Those with severe and fatal outcomes after a third dose tested positive a median of 44 days (28–56) after vaccination with BNT162b2 and 61 days (33–101) after vaccination with CoronaVac (table 1). Severe disease and death occurred predominantly in the unvaccinated population (figure 2).

We found some protection against mild or moderate disease from two doses of either CoronaVac or BNT162b2 in adults aged 20–59 years (table 2). Both vaccines were estimated to have high effectiveness against severe disease in adults aged 20–59 years, in whom vaccine effectiveness was estimated to be 96·3% (95% CI 94·9–97·3) for two doses of BNT162b2 and 91·7% (88·7–94·0) for two doses of CoronaVac (table 2). The difference in vaccine effectiveness was greater for older adults, with higher effectiveness among adults aged 60 years or older who received two doses of BNT162b2 (89·3% [86·6–91·6]) compared with those who received two doses of CoronaVac (69·9% [64·4–74·6]). When disaggregated further by age, we estimated that vaccine effectiveness was 91·1% (86·9–94·0) for two doses of BNT162b2 and 79·3% (71·8–85·0) for two doses of CoronaVac in those aged 60–69 years, reducing to 86·9% (80·5–91·3%) for two doses of BNT162b2 and 58·2% (45·1–68·2) for two doses of CoronaVac among those aged 80 years or older (table 2). Findings were similar for death; in adults aged 80 years or older two doses of BNT162b2 offered a higher level of protection against fatal disease (90·3% [84·9–93·9%]) compared with two doses of CoronaVac (63·0% [50·3–72·5]).

We compared the two-dose schedules of both vaccines and found differences between BNT162b2 and CoronaVac for mild disease in younger adults (relative vaccine effectiveness of BNT162b2 *vs* CoronaVac 11·5% [95% CI 0·4–21·3]), but we could not generate robust relative vaccine effectiveness estimates for mild disease in older age groups. Compared with CoronaVac, two doses of BNT162b2 offered better protection against severe or fatal disease in adults younger than 60 years (relative vaccine effectiveness 52·3% [95% CI 29·8–67·8%]) and in those aged 60 years or older (59·8% [51·1–67·1]). Findings were similar for death in those aged 20–59 years

	Mild or moderate disease (n=5566)*	Severe or fatal disease (n=8875)	Fatal disease (n=6866)
Age, years			
20–49	3198 (57·5%)	170 (1·9%)	81 (1·2%)
50–69	1620 (29·1%)	1214 (13·7%)	764 (11·1%)
≥70	748 (13·4%)	7491 (84·4%)	6021 (87·7%)
Sex			
Male	2383 (42·8%)	5322 (60·0%)	4152 (60·5%)
Female	3183 (57·2%)	3553 (40·0%)	2714 (39·5%)
Vaccination status†			
No doses	1402 (25·2%)	6413 (72·3%)	5204 (75·8%)
One dose			
BNT162b2	157 (2·8%)	126 (1·4%)	81 (1·2%)
CoronaVac	227 (4·1%)	1143 (12·9%)	794 (11·6%)
Two doses			
BNT162b2	2169 (39·0%)	242 (2·7%)	149 (2·2%)
CoronaVac	1274 (22·9%)	870 (9·8%)	596 (8·7%)
Three doses			
BNT162b2	125 (2·2%)	28 (0·3%)	16 (0·2%)
CoronaVac	212 (3·8%)	53 (0·6%)	26 (0·4%)
Median number of days between last vaccine dose and positive SARS-CoV-2 test result‡			
One dose			
BNT162b2	27 (21–35)	21 (18–31)	21 (17–29)
CoronaVac	29 (21–35)	22 (17–31)	22 (17–32)
Two doses			
BNT162b2	181 (150–216)	167 (76–209)	172 (92–217)
CoronaVac	179 (146–209)	125 (47–166)	122 (47–164)
Three doses			
BNT162b2	31 (20–48)	44 (28–56)	50 (43–70)
CoronaVac	39 (25–66)	61 (33–101)	65 (32–106)

Data are n (%) or median (IQR). Includes confirmed COVID-19 cases in Hong Kong classified as having mild or moderate disease between Dec 31, 2021, and Feb 15, 2022; and severe or fatal disease or fatal disease between Dec 31, 2021 and 16 March 2022. *Number of mild or moderate cases occurring before Feb 16, 2022, due to changes in admission criteria. †Number of doses plus 14-day lag. ‡Median time since vaccination among those for whom 14 days had passed since latest dose.

Table 1: **Participant characteristics**

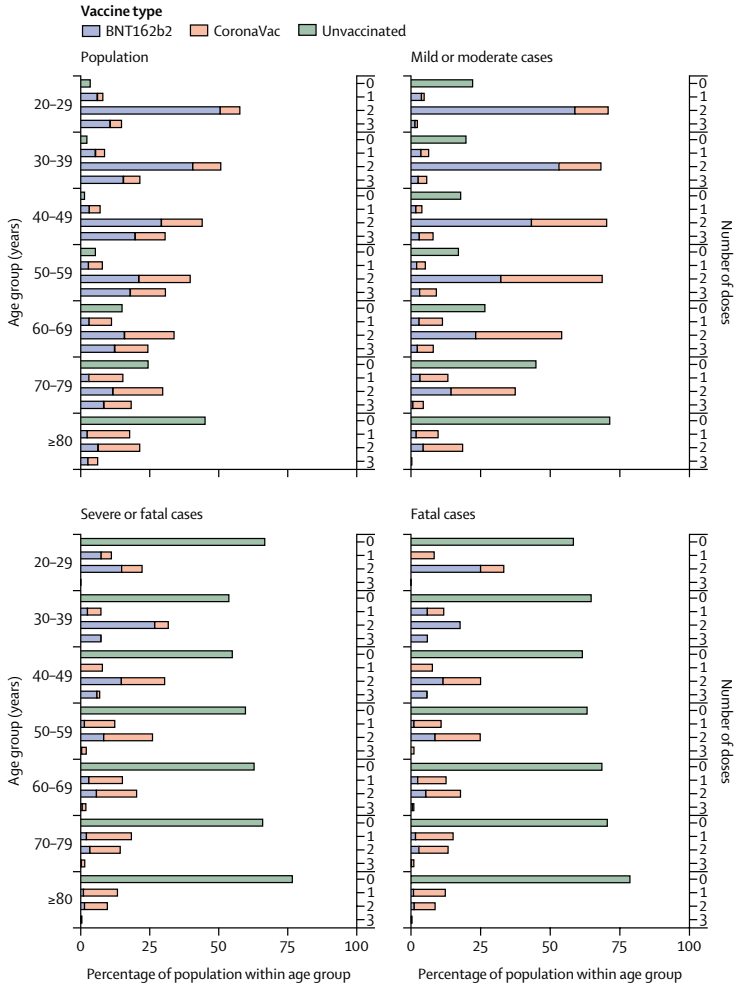


Figure 2: **Vaccine status, age group, and vaccine type**

(relative vaccine effectiveness 49·8% [15·5–70·5]) and in those aged 60 years or older (62·5% [52·9–70·3]).

We estimated that three recent doses of any vaccine (median time between third dose and onset 44 days for BNT162b2 and 61 days for CoronaVac; table 1) offered very high protection against severe disease (97·9% [95% CI 97·3–98·4]) and death (98·6% [98·0–99·0]), which was sustained within all age groups (appendix p 5). Vaccine effectiveness estimates were similar for both vaccines against severe and fatal outcomes (table 2). We estimated three doses of BNT162b2 to have a vaccine effectiveness of 73·5% (66·6–79·2) against mild or moderate disease in adults aged 20–59 years, whereas for three doses of CoronaVac we estimated the vaccine effectiveness to be 51·0% (39·6–60·4) against the same outcome (table 2). Vaccine effectiveness estimates that were calculated and adjusted for each week of the study period, rather than calendar day; or which considered a 7 day rather than 14 day duration between vaccination and immune response, yielded qualitatively similar vaccine effectiveness results but often with less precision, particularly for one-dose schedules (appendix pp 6–7).

We estimated the relative effect of three doses versus two doses of each vaccine type (table 3). For mild or moderate disease we found an additional benefit of a third dose of BNT162b2 in adults aged 20–59 years (relative vaccine effectiveness 59·8% [95% CI 49·7–68·1]) and in adults aged 60 years or older (71·6% [55·6–82·8%]) who had previously received two doses of BNT162b2 (table 3). A third dose of CoronaVac also increased protection in adults aged 20–59 years (35·7% [22·1–47·3]) and in adults aged 60 years or older (46·9%

	One dose		Two doses		Three doses	
	BNT162b2	CoronaVac	BNT162b2	CoronaVac	BNT162b2	CoronaVac
Mild or moderate disease						
20–59 years	39·9% (24·8–52·3)	32·7% (14·4–47·6)	35·1% (26·6–42·5)	25·1% (14·7–34·3)	73·5% (66·6–79·2)	51·0% (39·6–60·4)
≥60 years	None*	None*	None*	None*	70·2% (53·3–82·0)	32·4% (8·3–51·0)
Severe or fatal disease						
20–59 years	95·4% (90·7–98·1)	74·8% (63·7–82·8)	96·3% (94·9–97·3)	91·7% (88·7–94·0)	98·6% (97·5–99·3)	98·8% (97·5–99·5)
60–69 years	70·0% (51·8–82·0)	54·2% (36·4–67·3)	91·1% (86·9–94·0)	79·3% (71·8–85·0)	98·9% (97·3–99·6)	97·4% (95·2–98·7)
70–79 years	72·2% (56·7–82·6)	29·2% (7·4–46·1)	89·8% (85·1–93·1)	74·3% (66·5–80·3)	99·0% (97·4–99·7)	95·4% (92·2–97·4)
≥80 years	75·0% (61·1–84·2)	39·0% (20·9–53·0)	86·9% (80·5–91·3)	58·2% (45·1–68·2)	97·1% (93·8–98·7)	97·3% (94·9–98·7)
Death						
20–59 years	96·7% (90·9–99·2)	78·2% (64·9–86·9)	96·8% (95·1–98·0)	93·3% (89·9–95·6)	99·2% (97·9–99·7)	99·4% (98·1–99·9)
60–69 years	77·6% (59·9–88·4)	65·6% (49·8–76·8)	92·7% (88·6–95·4)	84·3% (77·8–89·0)	99·0% (97·2–99·8)	99·0% (97·3–99·8)
70–79 years	80·5% (66·3–89·2)	45·3% (25·1–60·3)	92·3% (88·0–95·2)	76·7% (68·5–82·8)	99·4% (97·9–99·9)	97·0% (94·2–98·6)
≥80 years	78·7% (65·5–87·0)	44·8% (26·9–58·4)	90·3% (84·9–93·9)	63·0% (50·3–72·5)	97·5% (94·2–99·0)	97·9% (95·7–99·1)

Data are effectiveness (95% CI). *No evidence of protection based on a negative or very small positive point estimate and wide CIs.

Table 2: **Vaccine effectiveness by dose and vaccine type in all ages and within age categories against COVID-19**

	BNT162b2	CoronaVac
Mild or moderate disease		
20–59 years	59·8% (49·7–68·1)	35·7% (22·1–47·3)
≥60 years	71·6% (55·6–82·8)	46·9% (29·6–60·6)
Severe or fatal disease		
20–59 years	60·1% (24·2–81·0)	85·2% (67·2–94·4)
60–69 years	84·5% (62·8–94·8)	85·6% (72·7–93·1)
70–79 years	88·3% (69·5–96·6)	76·9% (63·9–86·0)
≥80 years	64·9% (29·3–84·4)	87·9% (79·5–93·3)
Mortality		
20–59 years	71·2% (25·5–91·6)	91·0% (61·0–97·9)
60–69 years	84·2% (54·1–96·3)	92·5% (79·3–98·2)
70–79 years	90·0% (66·5–98·4)	82·6% (68·6–91·5)
≥80 years	61·8% (16·4–84·9)	88·6% (79·1–94·4)

Data are effectiveness (95% CI).

Table 3: **Relative vaccine effectiveness of three doses versus two doses of BNT162b2 and CoronaVac against COVID-19**

[29·6–60·6]) who had received two doses of CoronaVac (table 3). For severe or fatal disease we found an additional benefit of a third dose in adults of all ages for both vaccine types, with a relative vaccine effectiveness of 64·9% (29·3–84·4) for three versus two doses of BNT162b2, and 87·9% (79·5–93·3%) for three versus two doses of CoronaVac among those aged 80 years or older (table 3). Additional protection against death was offered by a third dose in all ages for both vaccines (table 3).

Discussion

We used detailed population-level data on the vaccination programme in Hong Kong and individual-level COVID-19 case data to estimate vaccine effectiveness of one, two, and three doses of BNT162b2 and CoronaVac vaccines in a largely infection-naive population during the fifth wave of COVID-19 in Hong Kong. Two or three doses of BNT162b2 or three doses of CoronaVac provided a very high level of protection against severe disease and death in all ages. We found a reduction in vaccine effectiveness among two-dose CoronaVac recipients, in particular for those aged 80 years or older. Some protection against mild or moderate disease was restored with third doses for both vaccines, but we were only able to estimate vaccine effectiveness for a shorter period since administration of third vaccine doses, and it is unclear how long this protection will last.

A case fatality rate of over 9% was observed in the older than 75 years throughout the study period. Although the precise relationship between immune response and clinical outcome is uncertain, the Hong Kong population had little pre-existing naturally or vaccine-derived humoral immunity to the omicron sublineage BA.2 before the beginning of the fifth wave.[21] Previous SARS-COV-2 infection has been shown to reduce fatality due to delta or omicron by approximately half (hazard ratio 0·47 [95% CI 0·32–0·68]) in vaccinated individuals and approximately five times (0·18 [0·06–0·57]) in unvaccinated individuals.[22] Therefore, the high death rates observed in Hong Kong might be at least partly attributed to the older population remaining largely unvaccinated and infection-naive, combined with health-system congestion. Furthermore, because available data only identified those who died within 28 days of testing positive, deaths from other causes in which COVID-19 disease was incidental or contributory could also have been included within these estimates. In the hospitalisation data used in our study, we found few serious or critical but non-fatal cases. We expect that this finding was a consequence of hospital overload and triage, whereby perhaps only the most serious cases were

admitted to ICU or EMCO facilities, but considering the magnitude of health-system disruption we cannot exclude information bias. We therefore applied a broad definition of severe case to account for these variations.

Almost all sequenced SARS-CoV-2 isolates during Hong Kong's fifth wave were of the omicron sublineage BA.2.[19,23,24] Our overall findings are largely consistent with existing vaccine effectiveness evidence against this sublineage. A study[25] in Qatar estimated that third-dose vaccine effectiveness for BNT162b2 against BA.2 was 43·7% (95% CI 36·5–50·0) in the first month and begins to decline again in the following weeks, with substantially improved protection against severe outcomes (6-week vaccine effectiveness 90·9% [78·6–96·1]). Similarly, a study of vaccine effectiveness in the USA[26] estimated vaccine effectiveness of two doses of any mRNA vaccine against severe omicron disease, defined as COVID-19 requiring invasive mechanical ventilation or in-hospital death, to be 79% (66–87), a median of 256 days after the second dose, and three-dose vaccine effectiveness to be 94% (88–97), a median of 60 days after the third dose.

Despite the overall consistency between our results and those presented in other studies, vaccine effectiveness could have been overestimated in our study. Reasons for vaccine hesitancy in Hong Kong have varied throughout the pandemic; however, hesitancy has typically been most prominent among adults older than 60 years, and associated with underlying health conditions.[27] Healthy vaccinee bias, by which vaccine recipients are healthier than their unvaccinated peers, might inflate the estimates in this setting. We could not formally assess this hypothesis with available data but our estimates are similar to those of other studies using alternative designs and we anticipate the magnitude of overestimation is unlikely to be substantial.[12,25] To address potential differences between vaccinated and unvaccinated cohorts, we also estimated a relative vaccine effectiveness of three versus two doses of each vaccine type; because individuals within these cohorts all chose to be vaccinated, they are more likely similar to each other in terms of baseline characteristics than their unvaccinated peers.[28] We found that a third dose of either vaccine provided additional protection, reiterating the public health value of a third dose for minimising the risk of severe disease and death but also for reducing health-system congestion and public concern.

Our finding that three doses of CoronaVac are needed for older adults to achieve high levels of protection is consistent with WHO recommendations for this group.[29] However, the estimates presented are likely to be affected by time since vaccination, in that typically more time has passed since administration of second than third doses, which have only been widely available in Hong Kong since the beginning of Jan, 2022. Data from Malaysia[15] comparing the duration of protection of the BNT162b2 and CoronaVac vaccines show more rapid waning of protection following CoronaVac after two doses, in particular for mild and moderate outcomes. Furthermore, two-dose immunogenicity data from Hong Kong indicate lower humoral and cellular responses following CoronaVac than with BNT162b2 vaccination but whether inactivated vaccines given in three-dose schedules will provide similar protection to the mRNA vaccines in the long term is unclear. However, evidence from our analyses that three doses of inactivated vaccine provide a high level of protection against severe COVID-19 disease, at least in the short term, is reassuring.[30]

Our study has several limitations. First, we used census data to construct the source population, but any differential population movement by vaccine status could affect the validity of our estimates. Furthermore, we estimated vaccine effectiveness in real-time and there might have been some delay in recording events, or missed unreported infections, which could underestimate case numbers and overestimate the denominator population-at-risk. Second, there are some differences in testing requirements by vaccine status, particularly for those required to regularly test because of occupation. However, we expect that estimates of vaccine effectiveness against severe outcomes will be only marginally susceptible to biases related to testing requirements. Third, we assumed that the second vaccine type matched the first, as per local guidelines, however a small number of people may have received mixed schedules. Fourth, our severe COVID-19 outcome included oxygen supplementation or therapy, which are coded using the 9th edition of the International Classification of Diseases, requiring medical staff to manually enter these procedures into electronic medical records with the potential for imperfect data entry and capture and under-ascertainment of these procedures. Finally, in Hong Kong there was a clear preference for the BNT162b2 vaccine in younger age groups and for CoronaVac in older adults. We have addressed this confounding in estimates presented by stratifying by age and adjusting estimates by 10-year age categories, sex, and calendar day. However, some residual confounding by age is possible in the vaccine platform-specific estimates and other factors might confound the relationship between vaccine status, type, and risk of infection that cannot be accounted for in this design.

Our findings indicate that two-dose schedules of both BNT162b2 and CoronaVac vaccines offer strong protection against severe disease and death; however, we found higher levels of protection among those who received two doses of BNT162b2 compared with those who received two doses of CoronaVac, particularly in older age groups. Three doses of either vaccine offered very high levels of protection for older adults against severe outcomes, with no differences observed across vaccine types. Our results show the importance of vaccination in an adult population that has acquired minimal protection from natural infection. Increasing uptake of third vaccine doses will be important,

particularly in older adults who have received two doses of CoronaVac. Further investigation of the durability of protection provided by both vaccines is warranted and planned.

Contributors

The study was designed by MEMM, JN, GML. and BJC. The underlying data were verified by YL, EHYL and MEMM, and data analyses were done by MEMM and YL. MEMM wrote the first draft of the manuscript, which was revised by JN and BJC. All authors interpreted data, provided critical review and revision of the text, and approved the final version of the manuscript.

Declaration of interests

BJC reports honoraria from AstraZeneca, Fosun Pharma, GlaxoSmithKline, Moderna, Pfizer, Roche, and Sanofi Pasteur. JN was previously employed by and owns shares in Sanofi. All other authors declare no competing interests.

Data sharing

Data on all vaccinations in Hong Kong by day and age are publicly available online (https://www.covidvaccine.gov.hk/en/dashboard). The clinical outcome data were extracted from the Hospital Authority database in Hong Kong and vaccine dose sequence for vaccinated cases were extracted from the eSARS COVID-19 surveillance database provided by the Centre for Health Protection. Restrictions apply to the availability of these data, used under license for this study. The hospitalisation and surveillance data were derived from records in the e-record system managed by the Hospital Authority and other databases by the Centre for Health Protection in Hong Kong and are restricted for reasons of patient consent. Data access can be discussed with the corresponding author or by approaching the Hospital Authority or Centre for Health Protection directly.

Acknowledgments

This project was supported by the Chinese Center for Disease Control and Prevention-sponsored COVID-19 Vaccines Evaluation Program (COVEP). BJC is supported by the Theme-based Research Scheme (T11-712/19-N) from the Research Grants Council from the University Grants Committee of Hong Kong, and an Research Grants Council Senior Research Fellowship (HKU SRFS2021-7S03). The authors thank the Hong Kong Government and Hospital Authority for the timely sharing of COVID-19 vaccination and case data. The authors thank Julie Au for administrative support.

References

1 Cowling BJ, Ali ST, Ng TWY, et al. Impact assessment of non-pharmaceutical interventions against coronavirus disease 2019 and influenza in Hong Kong: an observational study. *Lancet Public Health* 2020; **5:** e279–88.

2 Hong Kong Government. Latest situation of COVID-19 (as of April 15, 2022). 2022. https://www.chp.gov.hk/files/pdf/local_situation_covid19_en.pdf (accessed April 16, 2021).

3 Hong Kong Government. Third dose COVID-19 vaccination arrangements for persons under certain groups. 2021. Nov 3, 2021. https://www.info.gov.hk/gia/general/202111/03/P2021110300536.htm (accessed March 17, 2022).

4 Tsang J. Coronavirus: vaccine outreach teams to visit 'all Hong Kong care facilities by Friday', at home jabs to be offered to residents with mobility issues. *South China Morning Post* March 13, 2022. https://www.scmp.com/news/hong-kong/health-environment/article/3170304/coronavirus-vaccine-outreach-teams-visit-all-hong (accessed March 17, 2022).

5 Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. *BMJ* 2021; **373:** n1088.

6 Hall VJ, Foulkes S, Saei A, et al. COVID-19 vaccine coverage in health-care workers in England and effectiveness of BNT162b2 mRNA vaccine against infection (SIREN): a prospective, multicentre, cohort study. *Lancet* 2021; **397:** 1725–35.

7 Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of COVID-19 vaccines against the B.1.617.2 (delta) variant. *N Engl J Med* 2021; **385:** 585–94.

8 Higdon MM, Wahl B, Jones CB, et al. A systematic review of COVID-19 vaccine efficacy and effectiveness against SARS-CoV-2 infection and disease. *medRxiv* 2022; published online March 6. https://doi.org/10.1101/2021.09.17.21263549 (preprint).

9 Goldberg Y, Mandel M, Bar-On YM, et al. Waning Immunity after the BNT162b2 Vaccine in Israel. *N Engl J Med* 2021; **385:** e85.

10 Israel A, Merzon E, Schäffer AA, et al. Elapsed time since BNT162b2 vaccine and risk of SARS-CoV-2 infection: test negative design study. *BMJ* 2021; **375:** e067873.

11 Andrews N, Tessier E, Stowe J, et al. Duration of protection against mild and severe disease by COVID-19 vaccines. *N Engl J Med* 2022; **386:** 340–50.

12 Andrews N, Stowe J, Kirsebom F, et al. COVID-19 vaccine effectiveness against the omicron (B.1.1.529) variant. *N Engl J Med* 2022; published online March 2. https://doi.org/10.1056/NEJMoa2119451.

13 Collie S, Champion J, Moultrie H, Bekker L-G, Gray G. Effectiveness of BNT162b2 Vaccine against Omicron Variant in South Africa. *N Engl J Med* 2022; **386:** 494–96.

14 Cerqueira-Silva T, Katikireddi SV, de Araujo Oliveira V, et al. Vaccine effectiveness of heterologous CoronaVac plus BNT162b2 in Brazil. *Nat Med* 2022; published online Feb 9. https://doi.org/10.1038/s41591-022-01701-w.

15 Suah JL, Husin M, Keng Tok PS, et al. Waning COVID-19 vaccine effectiveness for BNT162b2 and CoronaVac in Malaysia: an observational study. *medRxiv* 2022; published online Jan 16. https://doi.org/10.1101/2022.01.15.22269326 (preprint).

16 Suryatma A, Anasi R, Hananto M, et al. Effectiveness of the inactivated COVID-19 vaccine (CoronaVac) in adult population in Bali, Indonesia. *medRxiv* 2022; published online Feb 4. https://doi.org/10.1101/2022.02.02.22270351 (preprint).

17 Jara A, Undurraga EA, González C, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. *N Engl J Med* 2021; **385:** 875–84.

18 Haas EJ, Angulo FJ, McLaughlin JM, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. *Lancet* 2021; **397:** 1819–29.

19 Mesfin Y, Chen D, Bond H, et al. Epidemiology of infections with SARS-CoV-2 omicron BA.2 variant in Hong Kong, January–March 2022. *medRxiv* 2022; published online April 14. https://doi.org/10.1101/2022.04.07.22273595 (preprint).

20 Sing C-W, Woo Y-C, Lee ACH, et al. Validity of major osteoporotic fracture diagnosis codes in the Clinical Data Analysis and Reporting System in Hong Kong. *Pharmacoepidemiol Drug Saf* 2017; **26:** 973–76.

21 Chen L-L. Syed MUA, Chan W-M, et al. Contribution of low population immunity to the severe omicron BA.2 outbreak in Hong Kong. *Nat Portf* 2022; published online April 14. https://doi.org/10.21203/rs.3.rs-1512533/v1 (preprint).

22 Nyberg T, Ferguson NM, Nash SG, et al. Comparative analysis of the risks of hospitalisation and death associated with SARS-CoV-2 omicron (B.1.1.529) and delta (B.1.617.2) variants in England: a cohort study. *Lancet* 2022; **399:** 1303–12.

23 Kok K-H, Wong S-C, Chan W-M, et al. Co-circulation of two SARS-CoV-2 variant strains within imported pet hamsters in Hong Kong. *Emerg Microbes Infect* 2022; **11:** 689–98.

24 Cheng VC-C, Ip JD, Chu AW-H, et al. Rapid spread of SARS-CoV-2 omicron subvariant BA.2 in a single-source community outbreak. *Clin Infect Dis* 2022; ciac203.

25 Chemaitelly H, Ayoub HH, AlMukdad S, et al. Duration of mRNA vaccine protection against SARS-CoV-2 omicron BA.1 and BA.2 subvariants in Qatar. *medRxiv* 2022; published online March 13. https://doi.org/10.1101/2022.03.13.22272308 (preprint).

26 Tenforde M, Self W, Gaglani M, et al. Effectiveness of mRNA vaccination in preventing COVID-19-associated invasive mechanical ventilation and death—United States, March 2021—January 2022. *MMWR Morb Mortal Wkly Rep* 2022; published online March 18. https://doi.org/10.15585/mmwr.mm7112e1.

27 Xiao J, Cheung JK, Wu P, Ni MY, Cowling BJ, Liao Q. Temporal changes in factors associated with COVID-19 vaccine hesitancy and uptake among adults in Hong Kong: serial cross-sectional surveys. *Lancet Reg Health West Pac* 2022; **23**: 100441.

28 McMenamin ME, Bond HS, Sullivan SG, Cowling BJ. Estimation of relative vaccine effectiveness in influenza: a systematic review of methodology. *Epidemiology* 2022; **33:** 334–45.

29 WHO. The Sinovac-CoronaVac COVID-19 vaccine: what you need to know. World Health Organization. Sept 2, 2021. https://www.who.int/news-room/feature-stories/detail/the-sinovac-covid-19-vaccine-what-you-need-to-know (accessed March 21, 2022).

30 Peng Q, Zhou R, Wang Y, et al. Waning immune responses against SARS-CoV-2 variants of concern among vaccinees in Hong Kong. *EBioMedicine* 2022; **77:** 103904.

7.2. Estudo chinês indica que dose de reforço da CoronaVac multiplica em 78 vezes os anticorpos neutralizantes

Uma pesquisa realizada em Pequim, na China, mostrou que a dose de reforço da CoronaVac aumentou quase 80 vezes o nível de anticorpos neutralizantes, elevando especificamente os anticorpos IgG das subclasses IgG1 e IgG3 – algo que ainda não tinha sido observado pela ciência. O trabalho foi publicado na revista Immunology e conduzido por pesquisadores do Centro de Controle e Prevenção de Doenças de Pequim e da Universidade Médica da Capital.

O estudo incluiu 174 adultos com idades entre 18 e 59 anos que receberam duas doses de CoronaVac. Destes, 158 receberam a terceira dose depois de um ano da imunização primária e foram acompanhados por 15 meses. Após 10 dias do reforço, a soroconversão atingiu 100% e se manteve assim nos meses seguintes. Já os anticorpos neutralizantes aumentaram 78 vezes, atingindo um pico de 290,6 após três semanas.

Os pesquisadores também avaliaram, pela primeira vez, as subclasses de anticorpos, que podem servir como importantes determinantes da eficácia da vacina. Os anticorpos neutralizantes são compostos principalmente por IgG, IgA e IgM, dentre os quais o IgG é o mais abundante e multifuncional. O IgG possui 4 subclasses distintas: IgG1, IgG2, IgG3 e IgG4.

Após a aplicação da dose de reforço, os níveis para IgG1 multiplicaram 21 vezes, aumentando de 21,7 para 458,4. Já os níveis para IgG3 subiram oito vezes, de 29,5 para 241,8. A proporção de IgG1 e IgG3 foi correlacionada ao alto nível de anticorpos capazes de neutralizar o vírus.

De acordo com os pesquisadores, já foi demonstrado que a resposta imune humoral de indivíduos recuperados da Covid-19 consiste principalmente em células B de memória produtoras de IgG1. "Ainda é preciso investigar se a imunização com vacinas inativadas [como a CoronaVac] está diretamente relacionada à presença de células B de memória."

Publicado em: 25/6/2022

Artigo

Received: 23 February 2022 | Accepted: 17 May 2022

DOI: 10.1111/imm.13531

ORIGINAL ARTICLE

immunology

The kinetics of IgG subclasses and contributions to neutralizing activity against SARS-CoV-2 wild-type strain and variants in healthy adults immunized with inactivated vaccine

Weixin Chen[1] | **Lichi Zhang**[1] | **Juan Li**[1] | **Shuang Bai**[1] | **Yali Wang**[1] | **Bing Zhang**[1] | **Qun Zheng**[2] | **Meng Chen**[1] | **Wei Zhao**[1] | **Jiang Wu**[1]

[1]Beijing Center for Disease Prevention and Control, Beijing, China

[2]Experimental Center for Basic Medical Teaching, School of Basic Medical Sciences, Capital Medical University, Beijing, China

Correspondence
Jiang Wu and Wei Zhao, Beijing Center for Disease Prevention and Control, No. 16 Hepingli Middle Street, Dongcheng District, Beijing 100013, China.
Email: wj81732@hotmail.com (J. W.) and zw830424@163.com (W. Z.)

Funding information
Beijing Municipal Science and Technology Commission, Grant/Award Number: Z211100002521014

Abstract

Neutralizing antibody is an important indicator of vaccine efficacy, of which IgG is the main component. IgG can be divided into four subclasses. Up to now, studies analysing the humoral response to SARS-CoV-2 vaccination have mostly focused on measuring total IgG, and the contribution of specific IgG subclasses remains elusive. The aim of this study is to investigate the kinetics of neutralizing antibodies and IgG subclasses, and to explore their relationships in people vaccinated with inactivated COVID-19 vaccine. We conducted a prospective cohort study in 174 healthy adults aged 18–59 years old who were administrated 2 doses of CoronaVac 14 days apart and a booster dose 1 year after the primary immunization, and followed up for 15 months. Blood samples were collected at various time points after primary and booster immunization. We used live SARS-CoV-2 virus neutralizing assay to determine neutralizing ability against the wild-type strain and 4 variants (Beta, Gamma, Delta and Omicron) and ELISA to quantify SARS-CoV-2 RBD-specific IgG subclasses. The results showed that the 2-dose primary immunization only achieved low neutralizing ability, while a booster shot can significantly enhance neutralizing ability against the wild-type strain, Beta, Gamma, Delta and Omicron variants. IgG1 and IgG3 were the most abundant serum antibodies, and IgG2 and IgG4 were hardly detected at any time. The ratio of IgG1/IgG3 was positively associated with the neutralization ability. The underlying mechanism requires further exploration.

KEYWORDS
IgG subclasses, neutralization ability, SARS-CoV-2 inactivated vaccine, variants

INTRODUCTION

Since 2019, the COVID-19 pandemic has been responsible for more than 6.18 million deaths [1]. As of April 5th, 2022, 35 COVID-19 vaccines have been approved for use worldwide with more than 11.25 billion administered doses [2, 3]. Among these vaccines, inactivated vaccines have been approved for use in more than 60 countries, and are the main vaccine type used in China. Compared with other vaccines, COVID-19 inactivated vaccines produced with traditional technology contain inactivated whole-virion SARS-CoV-2, therefore they maintain the structure of epitopes on surface antigens, and are easier to store and transport [4]. Evidence from real-world studies of inactivated vaccines in Chile [5], Brazil [6] and China [7] shows that a 2-dose vaccination schedule can effectively prevent the infection of COVID-19, and can offer greater protection against severe clinical outcomes.

Although vaccinations have markedly flattened COVID-19 epidemic curves, active cases continue to surge as multiple variants of the SARS-CoV-2 continue to emerge and spread worldwide. Currently 5 variants of concern (VOCs) have been defined by the WHO, including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529) [8]. All these VOCs have specific mutations within the spike regions [9], which may lead to escape from immunity induced by the prior infections or vaccinations, thereby potentially causing a large number of breakthrough infections [10, 11]. Although how new mutations can potentially affect the epidemic curves of the pandemic in the future is currently uncertain, the WHO still places high hopes on vaccination drive throughout world [3].

Since previous vaccination programs have faced the threat of circulating VOCs, booster immunization has become a standard practice to protect against variants, and has been started in more than 100 countries [3]. A preliminary evaluation has demonstrated that the additional dose could significantly reduce the number of new infections and symptomatic cases, and after the booster shot with CoronaVac the neutralization ability against the wild-type strain increased by more than 30 times [12–14]. However, the kinetics of neutralizing antibodies after primary and booster immunization are still unclear.

Neutralizing antibodies are mainly consisted of IgG, IgA and IgM, among which IgG is the most abundant and multifunctional component of neutralizing antibodies [15–17]. IgG consists of 4 distinct subclasses, defined by the structure of their constant regions, including IgG1, IgG2, IgG3 and IgG4. The IgG subclasses induced by vaccination can serve as important determinants of vaccine efficacy [18, 19]. However, up to now, studies analysing the humoral response to SARS-CoV-2 vaccination have mostly focused on measuring total IgG in the serum. Therefore, the contribution of specific IgG subclasses remains elusive.

The aim of the present study was to comprehensively describe the kinetics of neutralizing antibodies against the wild-type SARS-CoV-2 and 4 variants (Beta, Gamma, Delta and Omicron), to investigate the kinetics of IgG subclasses specific to the wild-type SARS-CoV-2 RBD, and to explore the correlation between IgG subclasses and neutralizing ability in a large-scale, long-term prospective cohort study of 174 healthy adults vaccinated with inactivated COVID-19 vaccines following primary and boost immunization.

MATERIALS AND METHODS

Study design, participants and serum collection method

Healthy, non-pregnant adults aged 18 to 59 years old were recruited. The main exclusion criteria included a history of SARS-CoV, SARS-CoV-2 or Middle East respiratory syndrome infection, a recent history (within 14 days before enrolment) of travel or exposure to infected individuals, axillary temperature > 37.0°C and reported allergy to any vaccine components. A complete list of exclusion criteria was included in the study protocol and approved by the Ethics Committee of Beijing Centre for Disease Prevention and Control (2020-28). Written informed consents were obtained from all participants both before enrolment and before the administration of the booster shot. All participants were tested for SARS-CoV-2 nucleic acid biweekly according to the study protocol.

The participants were administered two doses of CoronaVac (Sinovac Life Sciences, Beijing, China) which is an inactivated vaccine against COVID-19, at day 0 and day 14. A booster dose was given 12 months after the completion of the primary immunization. Blood samples were collected from the participants before vaccination and at 1, 3, 6 and 12 months after the second dose. The participants who received booster shots were randomly assigned to five groups, and their serum samples were collected on the 3rd, 7th, 10th, 14th and 21st days, respectively. The study design and sample collection schedule were summarized in Figure 1.

SARS-CoV-2 neutralization assay

Titres of neutralizing antibodies against live SARS-CoV-2 virus (wild-type strain: SARS-CoV-2/human/CHN/

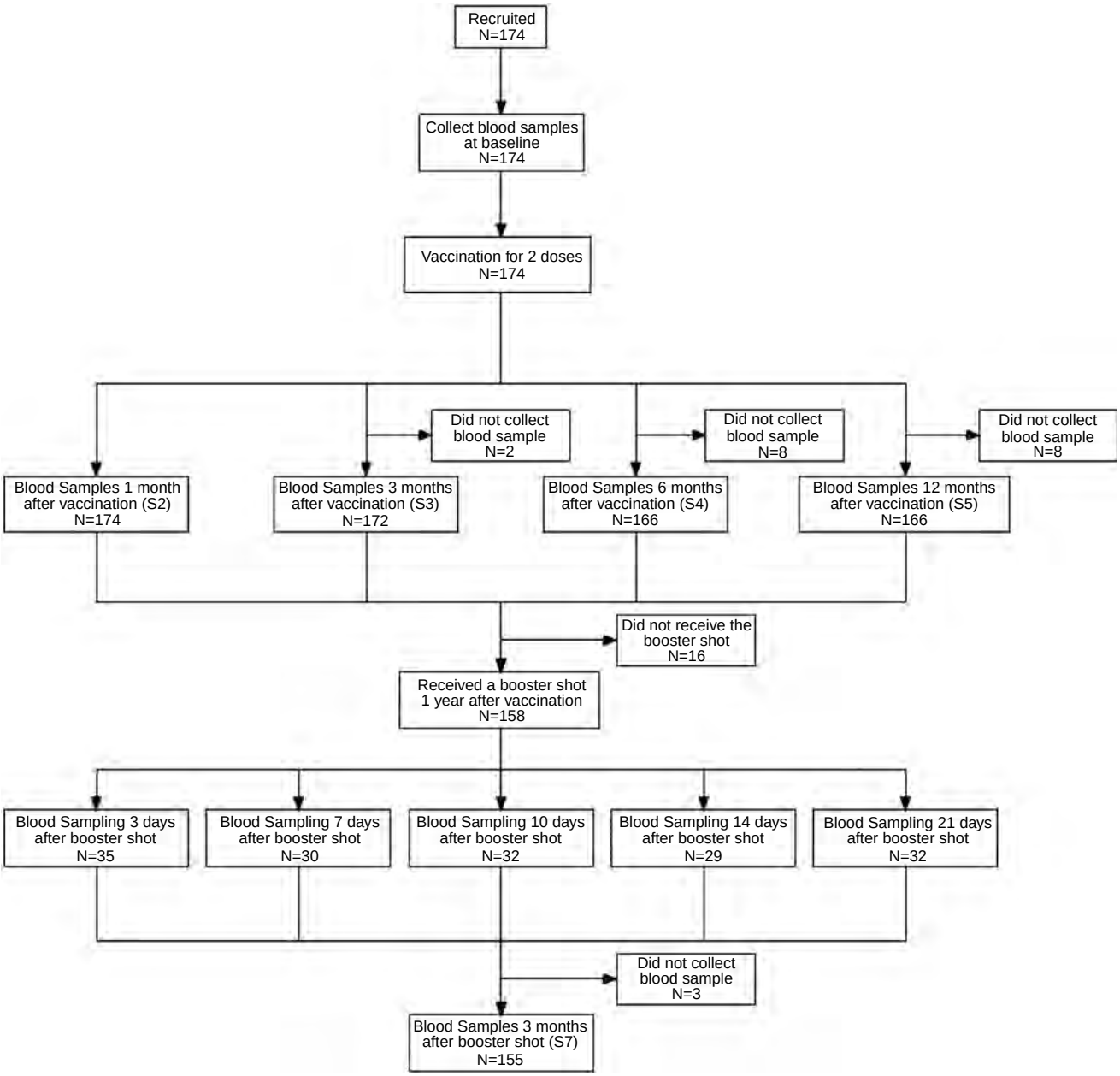


FIGURE 1 Study design and the sample collection

CN1/2020, Beta: EPI_ISL_2536954, Gamma: EPI_ISL_1060876, Delta: EPI_ISL_1911197, Omicron provided by Sinovac Life Sciences, Beijing, China) were quantified using the micro cytopathogenic effect assay. Briefly, the serum samples were incubated at 56°C for 30 min and serially diluted with the cell culture medium. The diluted samples were incubated in duplicate with 50 μl of SARS-CoV-2 virus suspension at 37.0°C for 2 h. Vero cells (1.0–2.0 × 10^5 cells/mL) were thereafter added to the suspension and incubated at 36.5°C for 5 days. The cytopathic effects were recorded and the neutralizing antibody titres were then calculated by the dilution number of 50% protective condition. Seroconversion threshold of the neutralizing antibodies was defined as a titre of 8 [20].

Anti-SARS-CoV-2 receptor binding domain (RBD) IgG subclasses assay

The anti-SARS-CoV-2 RBD-specific IgG subclasses serologic assay kit (ACROBiosystems) was used to measure the levels of IgG1, IgG2, IgG3 and IgG4 by an indirect enzyme linked immunosorbent assay (ELISA). The

microplate was pre-coated with the RBD of the spike protein. The sensitivity (lower detection limit) of SARS-CoV-2 RBD specific IgG1, IgG2, IgG3 and IgG4 monoclonal antibody was 10 ng/mL, 20 ng/mL, 0.2 ng/mL and 0.8 ng/mL, respectively. We used SARS-CoV-2 negative serum to validate its specificity. The specificity of IgG1, IgG2, IgG3 and IgG4 was 94.8%, 97.7%, 93.1% and 100%, respectively. The initial dilution of the sample was set to 1:20, since the dilution ratio of 1:20 can eliminate the background signal to the greatest extent while maintaining relatively high sensitivity. The cut-off value was 0.1, as calculated by 2.1 times the standard deviation of the OD values of a large number of SARS-CoV-2 negative serum. If the OD value/cut-off value (S/CO) was ≥1, then the test result was considered positive, whereas S/CO < 1 was negative. The corresponding level of IgG subclasses was calculated as S/CO × dilution folds. If saturated OD signals were observed, the samples were serially diluted until a negative test result was reached. The maximum dilution multiple of the positive test results was selected, and the corresponding OD value of the maximum dilution/cut-off × dilution multiple was the antibody level corresponding to the sample.

Statistical analysis

IgG subclass levels were presented as S/CO × dilution folds with 95% confidence intervals (CIs). The neutralizing antibody titres were presented as dilution folds. The geometric mean titres (GMTs) and 95% CIs were calculated with log values of the titres followed by subsequent antilog-transformation. Bonferroni's multiple comparison test was used to compare the titres of neutralizing antibodies against different strains. Mann–Whitney *U* test was used to compare the neutralizing antibody titres at different time points. Two-sided *p*-values < 0.05 were considered statistically significant. Statistical analyses were conducted with GraphPad Prism 8.0.1.

RESULTS

Demographic characteristics

A total of 174 participants were enrolled in this study. None of the participants was tested positive for SARS-CoV-2 nucleic acid until the end of the follow-up. The oldest participant was 59 years old, and the youngest was 22 years old. The mean age of the cohort was 39.7 years old. 43.7% of the participants were male and 56.3% were female.

Immunogenicity and antibody persistence after 2-dose primary immunization

Regarding the wild-type strain, the GMT (geometric mean titre) of the neutralizing antibodies reached the peak of 7.1 at 1 month after the primary immunization, with a seroconversion rate of 50.6% (Figure 2a and Table S1). At 3 months, the GMT decreased to 4.5, and the seropositivity rate decreased to 21.5%. Interestingly, at 6 months, the GMT increased to 5.5, and the seropositivity rate increased to 37.3%. At 12 months, the GMT decreased again to 4.6, and the seropositivity rate decreased to 25.9%.

The neutralization capacity against the variants was lower than that of the wild-type. At 1 month, the GMT was 2.2, 2.7, 2.4 and 2.0 for Beta, Gamma, Delta and Omicron variants, respectively. The seroconversion rate was 2.9%, 2.9%, 4.6% and 0.0% for Beta, Gamma, Delta and Omicron variants, respectively (Figure 2b–e and Table S1). The GMT and seropositivity rate against all the analysed variants did not change significantly until 12 months. After the 2-dose primary immunization, the GMT against the wild-type and all the variants strain did not reach the positive threshold of 1:8 at any time.

Immunogenicity and antibody persistence after the booster shot

A booster shot was administered to 158 healthy participants who completed the 2-dose primary immunization. Regarding the wild-type strain, on the 3rd day after the booster shot, the GMT (3.7) and seroconversion rate (22.9%) of the neutralizing antibodies were comparable to that of 12 months after the primary immunization. The GMT increased significantly thereafter. On the 7th day, the GMT increased to 37.3, and the seroconversion rate was 90% (27/30). The GMT continued to increase on the 10th day (158.2), 14th day (228.2), and reached a peak of 290.6 on the 21st day, thereafter decreased to 143.6 at 3 months (Figure 3a and Table S1). The seroconversion rate reached 100% at the 10th day and remained 100% thereafter.

The four variants showed similar trends with much lower GMTs ($p < 0.0001$). The peak GMTs of the four variants were reached on the 21st day, 52.5, 78.4, 46.0 and 24.0, respectively. At 3 months after the booster shot, the GMTs decreased significantly to 20.4, 37.5, 22.9 and 6.6; the seropositivity rates decreased to 80.6%, 92.9%, 83.9% and 42.6% for Beta, Gamma, Delta and Omicron variants, respectively (Figure 3b–e and Table S1). We used Bonferroni's multiple comparison test to compare

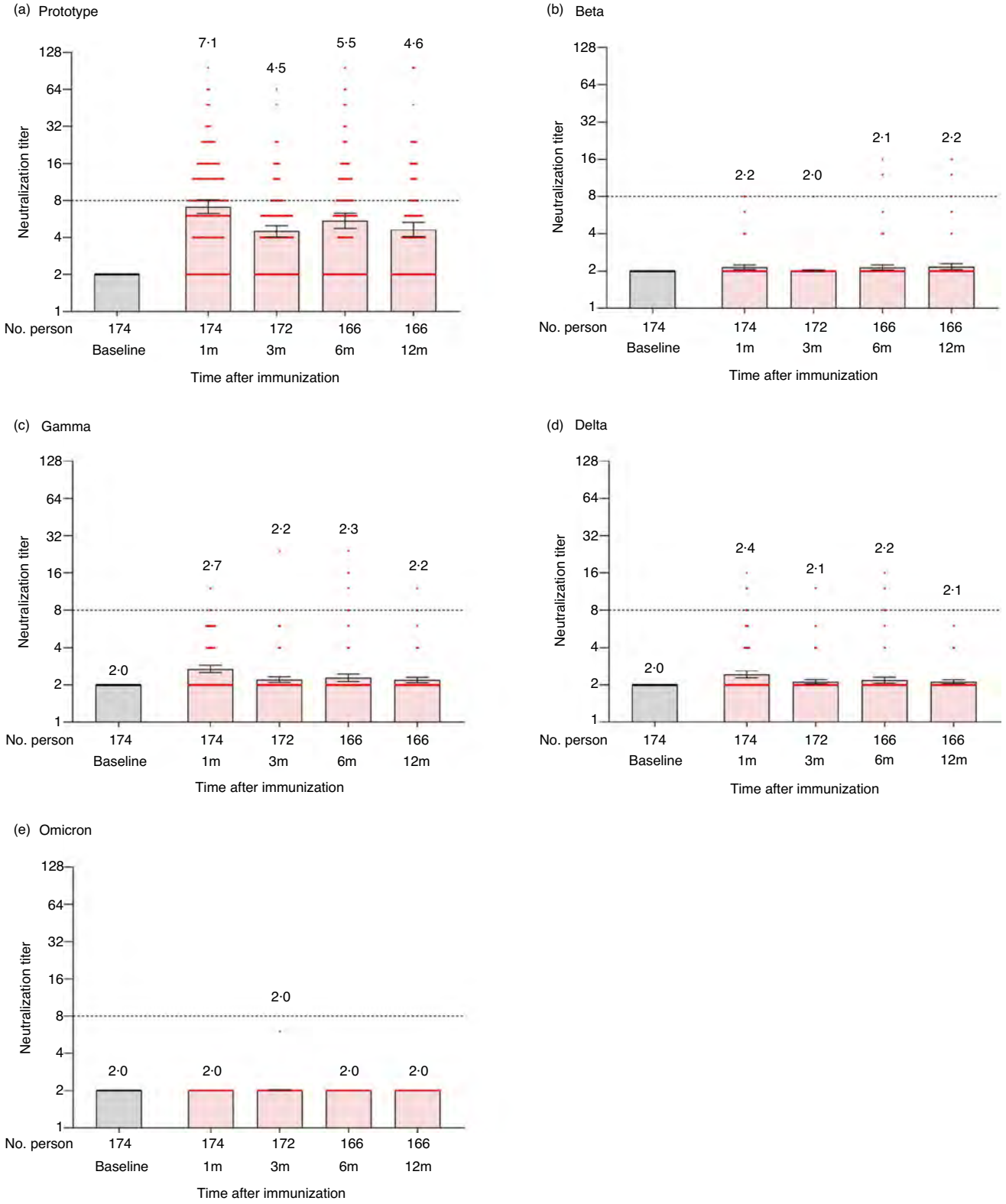


FIGURE 2 Neutralizing antibodies against the wild-type strain and variants of SARS-CoV-2 after 2-dose primary immunization. The results of neutralization assays against SARS-CoV-2 wild-type strain (a), Beta variant (b), Gamma variant (c), Delta variant (d), and Omicron variant (e). Each dot represents the neutralizing antibody titre of an individual. The numbers indicated above the bars are the geometric mean titres (GMT), and the error bars indicate the 95% confidence intervals (CI) of GMT. The dotted horizontal line represents the seropositivity threshold of 1:8. The titres lower than the limit of detection (1:4) are presented as half the limit of detection (1:2)

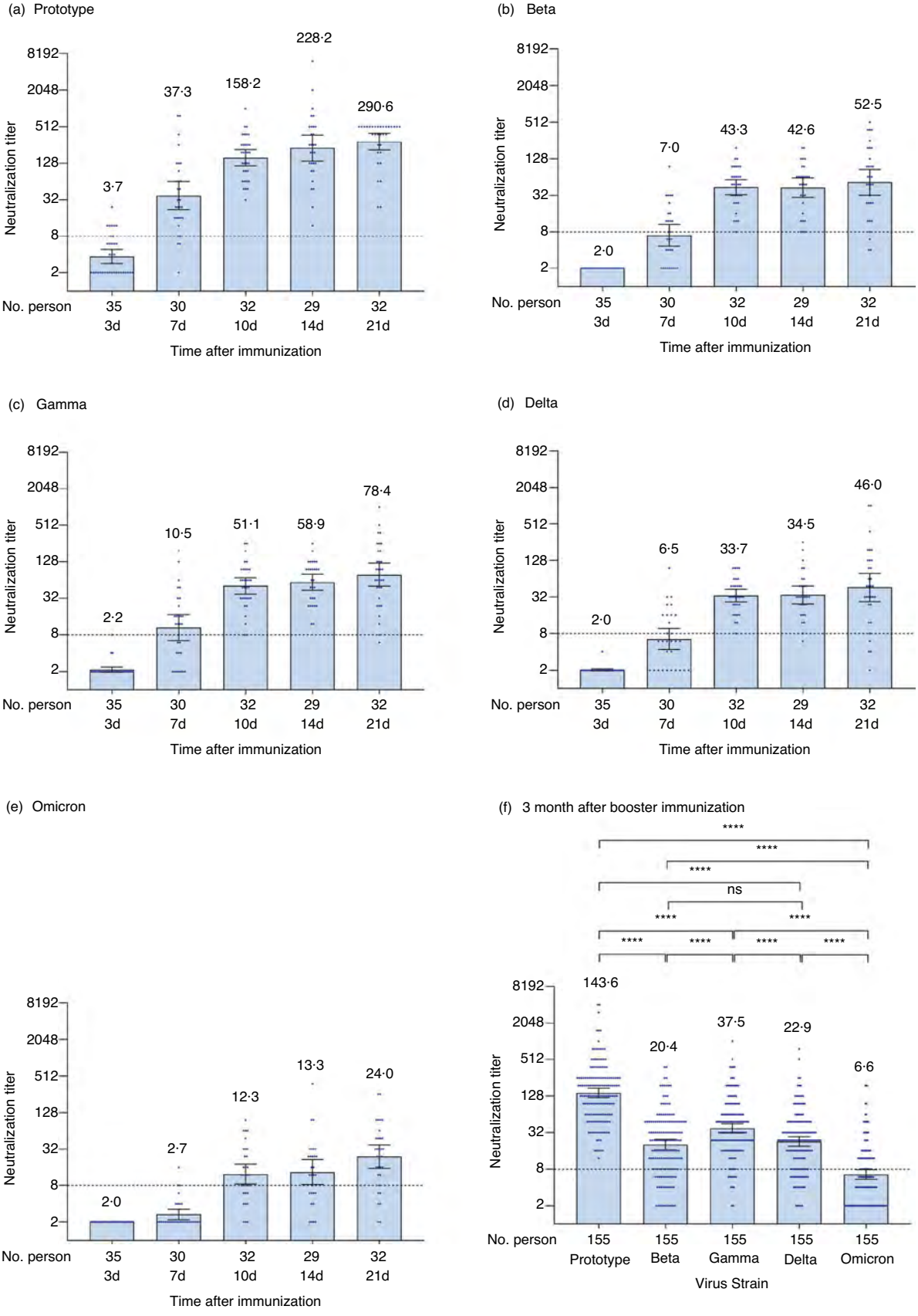


FIGURE 3 Legend on next page.

the neutralizing titres of the variants. Among the four variants, the GMT of the Gamma variant was the highest ($p < 0.0001$), and the GMT of the Omicron variant was the lowest ($p < 0.0001$). There were no significant differences observed in GMTs between Beta and Delta variants ($p > 0.9999$) (Figure 3f).

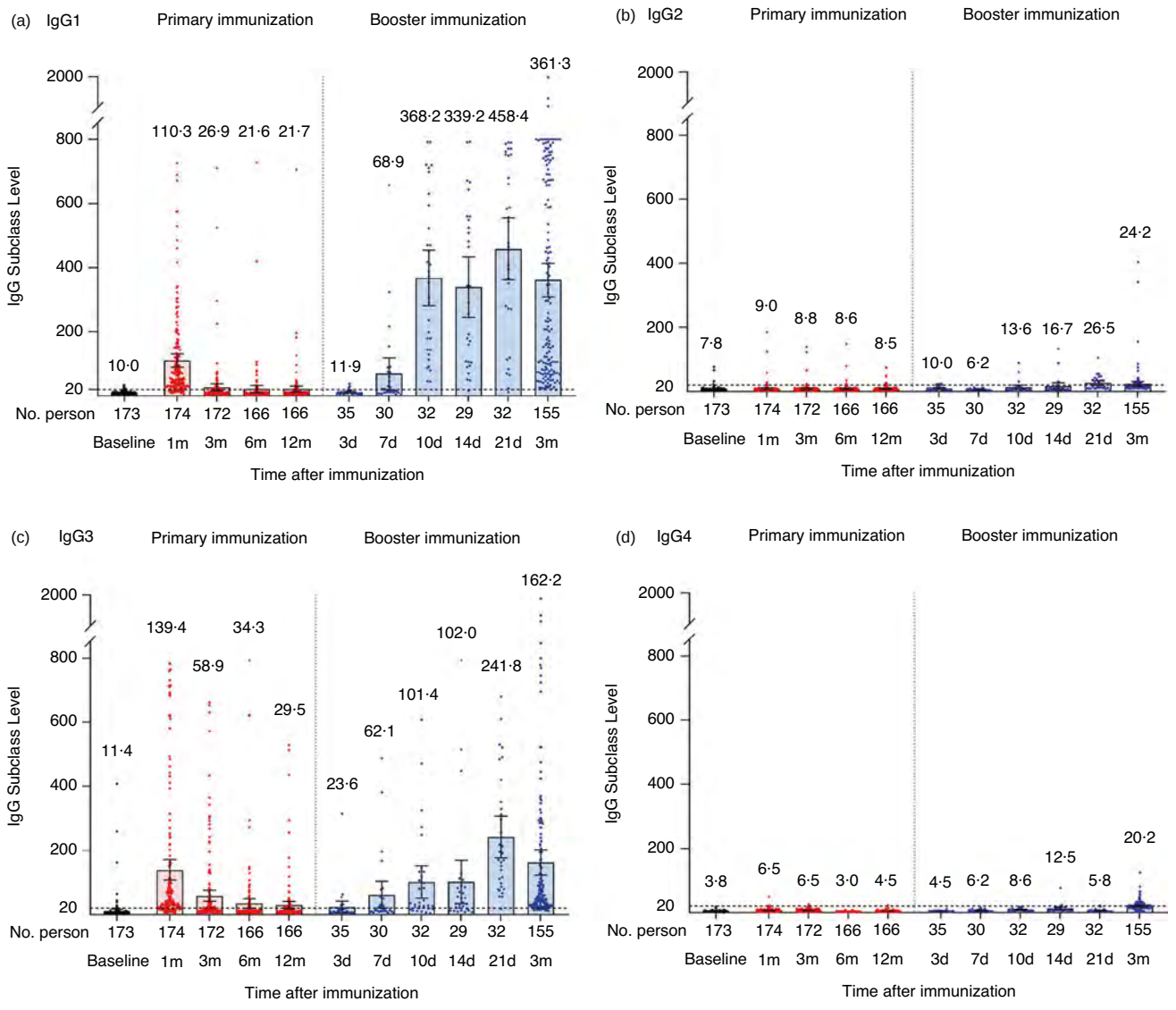


FIGURE 4 IgG subclasses at the different time points after 2-dose primary immunization and the booster shot. The levels of IgG1 (a), IgG2 (b), IgG3 (c) and IgG4 (d) after 2-dose primary immunization and the booster shot. Each dot represents the IgG subclass level of an individual. The numbers above the bars are the mean levels, and the error bars indicate the 95% CIs. The dotted horizontal line represents the seropositive threshold of 20

FIGURE 3 Neutralizing antibodies against the wild-type strain and variants of SARS-CoV-2 after the booster shot. (a–e) show the results of the different neutralization assays against SARS-CoV-2 wild-type strains (a), Beta variant (b), Gamma variant (c), Delta variant (d), and Omicron variant (e), while (f) shows the results at 3 months for the 5 strains. Each dot represents the neutralizing antibody titre of an individual. The numbers above the bars are GMTs, and the error bars indicate the 95% CIs of GMT. The dotted horizontal line represents the seropositivity threshold of 1:8. The titres lower than the limit of detection (1:4) are presented as half the limit of detection (1:2). Bonferroni's multiple comparison test was used to compare the titres of neutralizing antibodies against different strains in (f). ***$p < 0.001$, ****$p < 0.0001$, ns: not statistically significant. No multiple comparison adjustment has been done

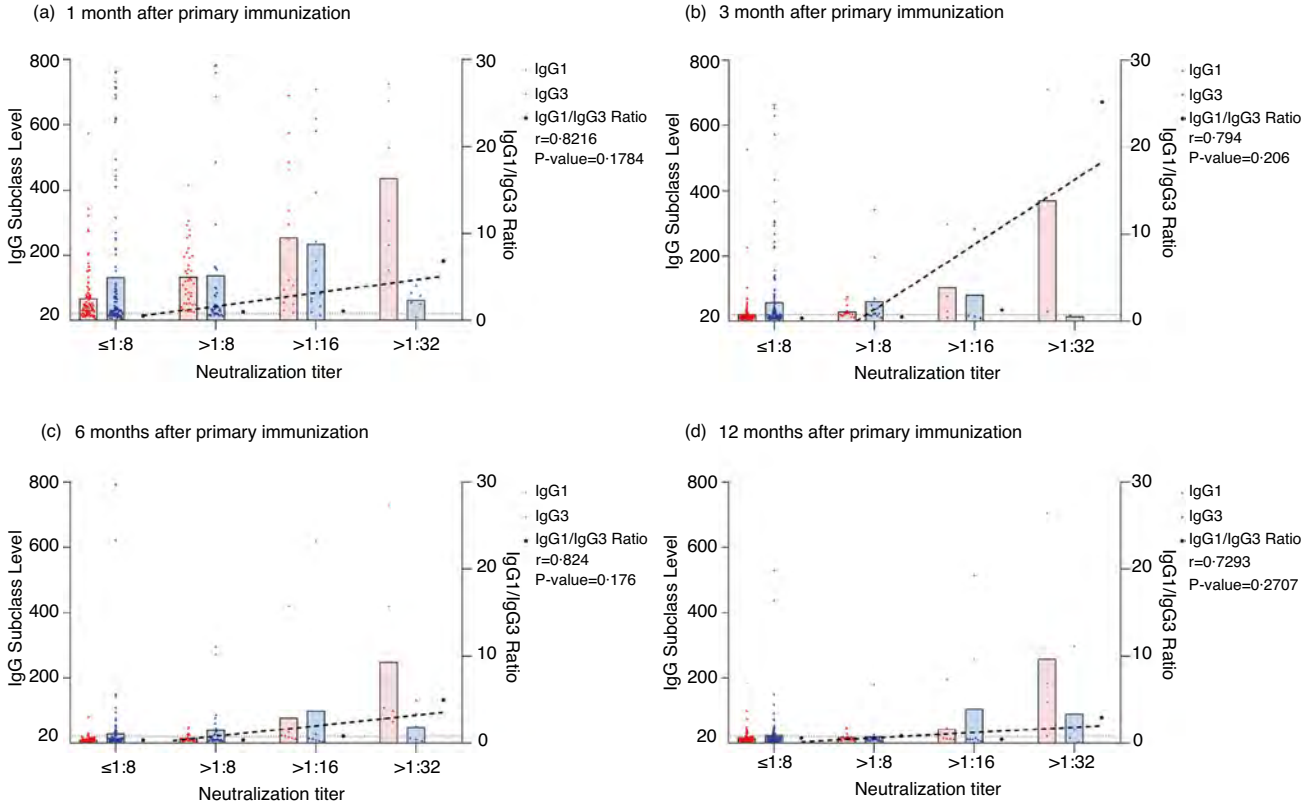


FIGURE 5 IgG1 and IgG3 distribution after 2-dose primary immunization. IgG1, IgG3 and IgG1/IgG3 ratio was grouped by titres of the neutralizing antibodies at 1 month (a), 3 months (b), 6 months (c) and 12 months (d) after 2-dose primary immunization. The dotted line represents the linear trend between the IgG1/IgG3 ratio and titres of the neutralizing antibodies. Based on each individual's neutralizing antibody titre, the seropositive threshold of 1:8, and the serial dilution multiplier of 2 in the neutralizing assay, the participants were divided into several mutually exclusive groups

SARS-CoV-2 RBD-specific IgG subclasses after 2-dose primary immunization and booster shot

ELISA analysis revealed that IgG1 and IgG3 were the most abundant subclasses (Figure 4). At 1 month after the 2-dose primary immunization, the mean levels of IgG1 and IgG3 were 110.3 and 139.4, respectively. At 3 months, both IgG1 and IgG3 levels decreased rapidly. After that, the level of IgG1 reached the plateau phase, and the level of IgG3 gradually decreased. At 12 months, the mean levels of IgG1 and IgG3 decreased to 21.7 and 29.5, respectively. On the 3rd day after the booster shot, the mean levels of IgG1 and IgG3 were comparable to that of 12 months after the primary immunization. Subsequently, IgG1 and IgG3 levels increased rapidly, especially that of IgG1. The mean levels of IgG1 and IgG3 reached a peak on the 21st days after the booster shot (458.4 and 241.8, respectively) and decreased at 3 months (361.3 and 162.2, respectively).

Relationship between IgG1, IgG3 subclasses and neutralizing antibodies

There was no correlation observed between the titres of the neutralizing antibodies and level of IgG1 or IgG3 individually (Table S2). We divided the participants into groups based on their neutralizing titres, that is, ≤8, 8–16, 16–32, 32–64, etc. The titres of the neutralizing antibodies showed a positive correlation with IgG1/IgG3 ratio in general. After the primary immunization, although the correlation was not linear, the correlation coefficients reached 0.8216 ($p = 0.1784$), 0.794 ($p = 0.206$), 0.824 ($p = 0.176$) and 0.7293 ($p = 0.2707$) at 1, 3, 6 and 12 months, respectively (Figure 5). After the booster shot, the titres of the neutralizing antibodies exhibited a strong linear relationship with the IgG/IgG3 ratio. At 1 month, the correlation coefficient was 0.776 ($p = 0.0236$) (Figure 6). At 3 months, the correlation coefficient was 0.9782 ($p = 0.0001$) (Figure 7a). As for the Beta, Gamma, Delta and Omicron variants, the correlation coefficients were 0.3296 ($p = 0.4703$), 0.6825

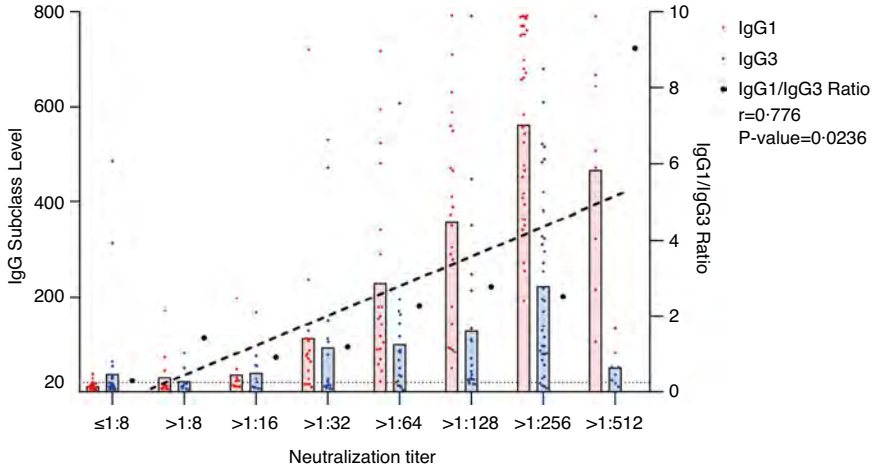


FIGURE 6 IgG1 and IgG3 distribution after the booster shot within 1 month. IgG1, IgG3 and IgG1/IgG3 ratio grouped by titres of the different neutralizing antibodies after the booster shot within 1 month. The dotted line reprints the linear trend between the IgG1/IgG3 ratio and titres of neutralizing antibodies. The grouping rationale similar to Figure 5 also applies here

(p = 0.0911), 0.3069 (p = 0.5031) and −0.0110 (p = 0.9842) at 3 months after the booster shot, respectively (Figure 7b–d).

DISCUSSION

In the present study, we showed the kinetics of neutralizing antibodies against the wild-type strain and variants after 2-dose primary immunization and a booster shot of inactivated vaccine. After the primary immunization, the GMT of neutralizing antibodies exhibited a moderate increase and reached the peak at 1 month. However, the GMT against the wild-type strain did not reach the threshold of 8 since most of the individuals had low titres of antibodies. The peak seroconversion rate was only 50.6% at 1 month. GMTs of the neutralizing antibodies against the variants were even lower. Therefore, regarding immunogenicity, the 2-dose primary immunization only achieved low neutralizing ability. After the booster shot, the GMT rapidly increased, and the seroconversion rate reached 100% within 10 days, for the wild-type strain, Beta, Gamma and Delta variants. At 3 months after the booster shot, the GMT against the wild-type strain was about 20.2 times the GMT at 1 month after the primary immunization (Table S3). This pattern of rapid immune response and persistence of antibodies was consistent with the characteristics of immune memory. This finding can largely dispel the concerns that inactivated vaccines cannot induce significant cell-mediated immune response, and that they can only result in weak immunogenicity with deficiency of long-time immune memory. Furthermore, efficacy of inactivated vaccine against the variants has been demonstrated indirectly due to increased immunogenicity after the booster shot.

This study showed that IgG1 and IgG3 were the most abundant IgG subclasses at all time points. This observation was consistent with Fraley et al.'s finding for mRNA vaccines [21] and Suthar et al.'s finding in patients naturally infected with SARS-CoV-2 [22]. More specifically, in this study IgG1 and IgG3 levels were similar after primary immunization, whereas IgG1 level was much higher than IgG3 level after the booster shot. It has been reported that the humoral immune response in individuals recovered from the natural infections of COVID-19 was primarily dominated by IgG1-producing memory B cells whereas the amount of IgG3-producing memory B cells was relatively low [23, 24]. Whether the humoral immune response after the booster immunization with SARS-CoV-2 inactivated vaccines might be directly related to the presence of memory B cells requires further verification.

Interestingly, the GMT and seropositivity rate of the neutralizing antibodies at 6 months after the primary immunization were higher than that at 3 months. Several previous studies have indicated that IgG subclass switching may substantially impact the titre of the neutralizing antibodies [25, 26]. It has been found that in infections with rubella and measles viruses, the affinity of IgG1 was higher than that of IgG3, and the affinity maturation time of IgG1 was later than that of IgG3 [27, 28]. We examined whether the relative amounts of IgG1 and IgG3 was correlated with the GMT of the neutralizing antibodies at different time points, and we noticed that a higher percentage of individuals with IgG1 > IgG3 is correlated with a higher GMT of the neutralizing antibody (Pearson correlation coefficient = 0.7718, p-value = 0.0089, Figure S3). More specifically, at 1 month after primary immunization, the percentage of individuals with IgG1/IgG3 > 2 is 39.7%, and the GMT of the neutralizing antibodies was relatively high. At 3 months, the percentage of individuals with IgG1/IgG3 > 2 decreased to 8.7%, and the GMT of the neutralizing antibodies decreased as well. At 6 months, the

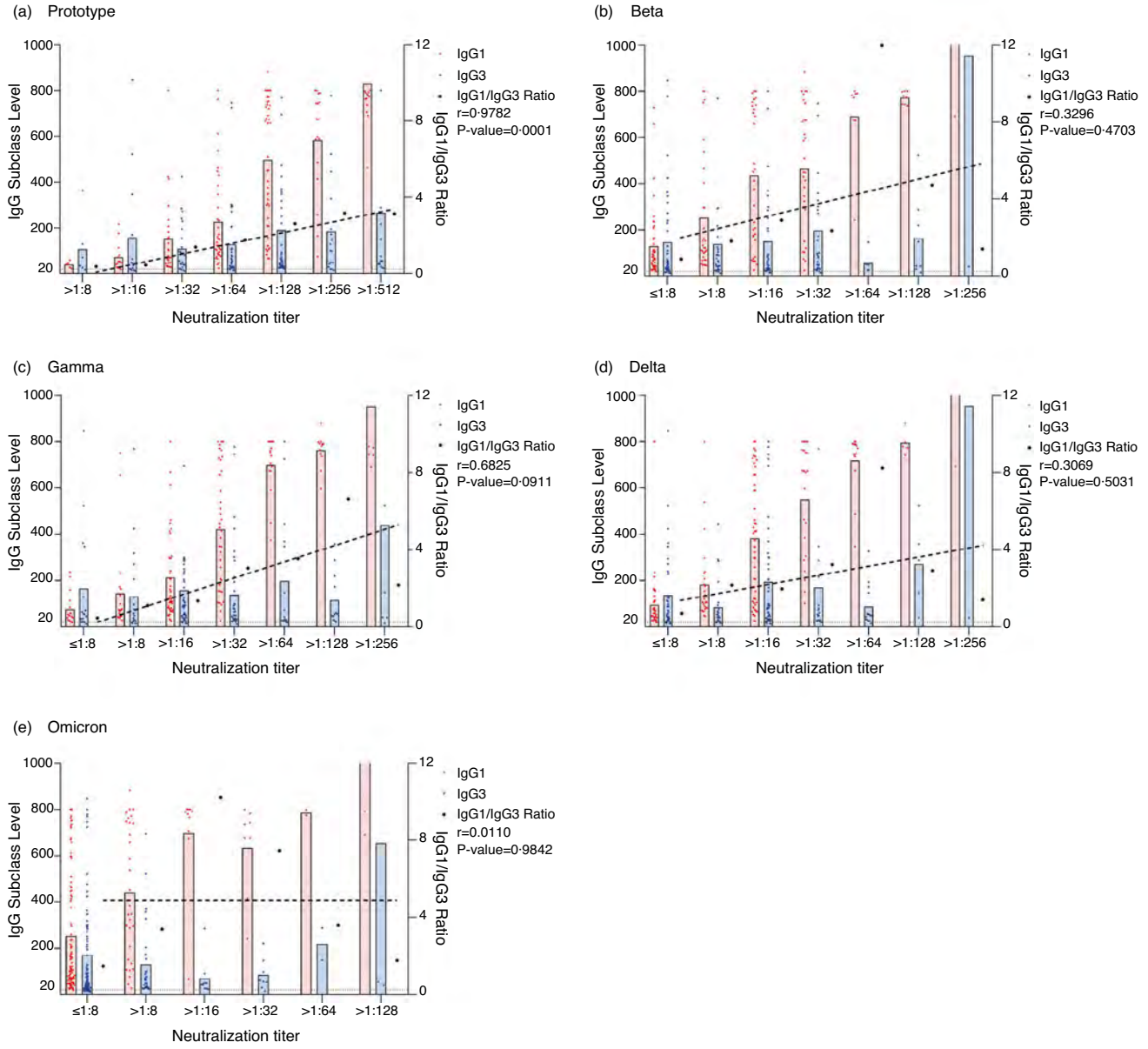


FIGURE 7 The association between IgG subclasses and neutralizing antibodies against the wild-type strain and variants at 3 months after the booster shot. IgG1, IgG3 and IgG1/IgG3 ratio grouped by the titres of neutralizing antibodies against the wild-type strain (a), Beta (b), Gamma (c), Delta (d) and Omicron(e) variants. The dotted line represents the linear trend between the IgG1/IgG3 ratio and the titres of different neutralizing antibodies. The grouping rationale similar to Figure 5 applies here

percentage of individuals with IgG1/IgG3 > 2 increased to 12.0%, and the GMTs of the neutralizing antibodies also increased (Table S3, Table S4, and Figure S1). Therefore, we speculate that IgG1 affinity maturation might have occurred between 3 to 6 months after the primary immunization with SARS-CoV-2 inactivated vaccines.

A positive correlation was observed between the ratio of IgG1/IgG3 and GMT of the neutralizing antibodies after we grouped the participants by their neutralizing titre. Individually, when the IgG1/IgG3 ratio was less than 5, the GMT of the neutralizing antibodies increased concomitantly with the IgG1/IgG3 ratio. When the IgG1/IgG3 ratio was 5 or higher, the GMT reached a plateau phase (Figure S2).

The neutralization assay was conducted in vitro to evaluate the potential inhibitory effect of antibodies against the virus. The inhibitory effect primarily depends on the selective binding of the Fab segment to the viral epitope, while the function of the Fc segment remains elusive. Three different types of Fc receptors (FcγRI, FcγRII and FcγRIII) can interact with IgG. In humans, the FcγRI is expressed on the surface of monocytes,

macrophages and dendritic cells, and can effectively bind to the monomeric IgG1 and IgG3 with high affinity [29]. Yates et al. have shown that the spike-specific IgG subclasses may contribute to COVID-19 disease severity through regulating potent Fc-mediated effector functions [30]. In addition, the different subclasses of vaccine-elicited antibodies may differentially recruit and activate innate immune effector cells expressing various IgG receptors on their surface, thereby significantly affecting the vaccine efficacy [31].

The present study has many strengths. First, we constructed a prospective cohort which covered a large sample size, a long follow-up time, and multiple time points. Second, the correlation between IgG1/IgG3 ratio and the GMT of the neutralizing antibodies is a novel discovery.

However, due to limited detection reagents, we were only able to investigate the RBD-specific IgG subclasses for the wild-type strain. Therefore, we cannot confidently draw the practical conclusions regarding the Beta, Gamma, Delta and Omicron variants. Further studies will examine IgG subclasses specific to variant strains.

In conclusion, the findings of this study have indicated that the 2-dose primary immunization of COVID-19 inactivated vaccine only achieved low neutralization ability. After administration of the booster shot, the neutralization ability against the wild-type strain and all variants significantly improved. IgG subclass switching affected neutralizing ability in people receiving COVID-19 inactivated vaccines. The ratio of IgG1/IgG3 was positively correlated with the titre of neutralizing antibody. The underlying mechanism is not yet clear, and requires further research.

AUTHOR CONTRIBUTIONS

Jiang Wu, Weixin Chen, Qun Zheng and Wei Zhao conceived the project. Jiang Wu, Shuang Bai, Juan Li, Bing Zhang and Meng Chen coordinated and performed the cohort study. Weixin Chen, Yali Wang and Bing Zhang performed the experimental measurements. Weixin Chen, Lichi Zhang and Jiang Wu analysed the data and wrote the manuscript with inputs from all authors.

ACKNOWLEDGEMENTS

We would like to thank all the participants who attended this study. In addition, we would like to thank Dr. Ying Sun (School of Basic Medical Science, Capital Medical University, Beijing, China) for discussion. This study was supported by the Beijing Municipal Science and Technology Commission (Z211100002521014).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

ORCID

Weixin Chen https://orcid.org/0000-0003-4519-2790

REFERENCES

1. https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (2022)
2. UNICEF. COVID-19 vaccine market dashboard (2022). Accessed 9 Feb 2022.
3. https://ourworldindata.org/covid-vaccinations (2022)
4. Thomas JK. Vaccines in Kuby immunology. 6th ed. England: W. H. Freeman and Company; 2006. p. 484.
5. Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. N Engl J Med. 2021;385:875–84.
6. Ranzani OT, Hitchings MDT, Dorion M, D'Agostini TL, de Paula RC, de Paula OFP, et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of COVID-19 in Brazil: test negative case-control study. BMJ. 2021;374:n2015.
7. Kang M, Yi Y, Li Y, Sun L, Deng A, Hu T, et al. Effectiveness of inactivated COVID-19 vaccines against COVID-19 pneumonia and severe illness caused by the B.1.617.2 (delta) variant: evidence from an outbreak in Guangdong, China. *SSRN*; published online Aug 5. 2021. https://ssrn.com/abstract=3895639
8. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/ (2022)
9. GISAID. https://www.gisaid.org/hcov19-variants/ (2022)
10. Li Q, Nie J, Wu J, Zhang L, Ding R, Wang H, et al. SARS-CoV-2 501Y.V2 variants lack higher infectivity but do have immune escape. Cell. 2021;184:2362–71e9.
11. Garcia-Beltran WF, Lam EC, St Denis K, Nitido AD, Garcia ZH, Hauser BM, et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. Cell. 2021;184:2523.
12. Bar-On YM, Goldberg Y, Mandel M, Bodenheimer O, Freedman L, Kalkstein N, et al. Protection of BNT162b2 vaccine booster against COVID-19 in Israel. N Engl J Med. 2021;385:1393–400.
13. Abu-Raddad LJ, Chemaitelly H, Butt AA, National Study Group for C-V. Effectiveness of the BNT162b2 COVID-19 vaccine against the B.1.1.7 and B.1.351 variants. N Engl J Med. 2021;385:187–9.
14. Zeng G, Wu Q, Pan H, Li M, Yang J, Wang L, et al. Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials. Lancet Infect Dis. 2021;22:483–95.
15. Robbiani DF, Gaebler C, Muecksch F, Lorenzi JCC, Wang Z, Cho A, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature. 2020;584:437–42.
16. Hartley GE, Edwards ESJ, Aui PM, Varese N, Stojanovic S, McMahon J, et al. Rapid generation of durable B cell memory

to SARS-CoV-2 spike and nucleocapsid proteins in COVID-19 and convalescence. Sci Immunol. 2020;5:eabf8891.
17. Chen X, Li R, Pan Z, Qian C, Yang Y, You R, et al. Human monoclonal antibodies block the binding of SARS-CoV-2 spike protein to angiotensin converting enzyme 2 receptor. Cell Mol Immunol. 2020;17:647–9.
18. Schur PH. IgG subclasses—a review. Ann Allergy. 1987;58: 89–96.
19. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol. 2014;5:520.
20. Han B, Song Y, Li C, Yang W, Ma Q, Jiang Z, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021;21:1645–53.
21. Fraley E, LeMaster C, Geanes E, Banerjee D, Khanal S, Grundberg E, et al. Humoral immune responses during SARS-CoV-2 mRNA vaccine administration in seropositive and seronegative individuals. BMC Med. 2021;19:169.
22. Suthar MS, Zimmerman MG, Kauffman RC, Mantus G, Linderman SL, Hudson WH, et al. Rapid generation of neutralizing antibody responses in COVID-19 patients. Cell Rep Med. 2020;1:100040.
23. de Campos-Mata L, Tejedor VS, Tacho-Pinot R, Pinero J, Grasset EK, Arrieta AI, et al. SARS-CoV-2 sculpts the immune system to induce sustained virus-specific naive-like and memory B-cell responses. Clin Transl Immunol. 2021;10:e1339.
24. Goel RR, Apostolidis SA, Painter MM, Mathew D, Pattekar A, Kuthuru O, et al. Distinct antibody and memory B cell responses in SARS-CoV-2 naïve and recovered individuals following mRNA vaccination. Sci Immunol. 2021;6:eabi6950.
25. Luo H, Jia T, Chen J, Zeng S, Qiu Z, Wu S, et al. The characterization of disease severity associated IgG subclasses response in COVID-19 patients. Front Immunol. 2021;12:632814.
26. Stephens DS, McElrath MJ. COVID-19 and the path to immunity. JAMA. 2020;324:1279–81.
27. Wilson KM, Di Camillo C, Doughty L, Dax EM. Humoral immune response to primary rubella virus infection. Clin Vaccine Immunol. 2006;13:380–6.
28. El Mubarak HS, Ibrahim SA, Vos HW, Mukhtar MM, Mustafa OA, Wild TF, et al. Measles virus protein-specific IgM, IgA, and IgG subclass responses during the acute and convalescent phase of infection. J Med Virol. 2004;72:290–8.
29. Neidich SD, Fong Y, Li SS, Geraghty DE, Williamson BD, Young WC, et al. Antibody Fc effector functions and IgG3 associate with decreased HIV-1 risk. J Clin Invest. 2019;129:4838–49.
30. Yates JL, Ehrbar DJ, Hunt DT, Girardin RC, Dupuis AP 2nd, Payne AF, et al. Serological analysis reveals an imbalanced IgG subclass composition associated with COVID-19 disease severity. Cell Rep Med. 2021;2:100329.
31. Nimmerjahn F, Ravetch JV. Antibody-mediated modulation of immune responses. Immunol Rev. 2010;236:265–75.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Chen W, Zhang L, Li J, Bai S, Wang Y, Zhang B, et al. The kinetics of IgG subclasses and contributions to neutralizing activity against SARS-CoV-2 wild-type strain and variants in healthy adults immunized with inactivated vaccine. Immunology. 2022. https://doi.org/10.1111/imm.13531

CoronaVac

O que a ciência comprova

7.3. Terceira dose da CoronaVac mantém elevada produção de anticorpos por seis meses, afirma estudo

Um estudo realizado na China voltou a mostrar que a terceira dose da CoronaVac é altamente eficaz para recuperar a imunidade, induzindo a produção rápida de anticorpos com duração prolongada. Após seis meses da dose de reforço, a positividade de anticorpos neutralizantes ainda era superior a 80%. O trabalho foi publicado no Journal of Infection e conduzido por pesquisadores da Faculdade de Medicina da Universidade de Xiamen.

Neste estudo de coorte prospectivo, 41 participantes receberam três doses da CoronaVac e tiveram amostras de sangue coletadas ao longo de 180 dias após a terceira dose. Os títulos de anticorpos neutralizantes, de anticorpos totais anti-RBD e de anticorpos IgG anti-Spike foram determinados para avaliar a resposta imune e sua duração.

Nove meses depois da segunda dose, a taxa de produção de anticorpos neutralizantes estava em 2,44%. Vale ressaltar que essa é uma condição comum a todas as vacinas contra a Covid-19, e está relacionada à dinâmica do SARS-CoV-2. Com a terceira dose, a soroconversão atingiu 100% em duas semanas, mantendo-se constante por mais dois meses, e então começou a diminuir lentamente, caindo para 80,49% em seis meses.

Para os anticorpos totais anti-RBD, a taxa de soroconversão estava em 39,02% após nove meses da segunda dose, atingindo o pico de 100% uma semana após a terceira dose e mantendo-se assim por seis meses. A resposta de anticorpos IgG anti-Spike após a dose de reforço foi semelhante.

"Os níveis de anticorpos neutralizantes são altamente preditivos de proteção imunológica. A importância dessas observações é que anticorpos neutralizantes em vacinados podem persistir, embora com uma taxa relativamente baixa de decaimento, e podem atuar como a primeira linha de defesa contra futuras infecções pela ômicron ou variantes futuras", afirmam os autores da pesquisa.

Publicado em: 9/6/2022

ARTICLE IN PRESS
JID: YJINF [m5G;June 12, 2022;8:23]
Journal of Infection xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Letter to the Editor

Is the fourth COVID-19 vaccine dose urgently needed? Revelation from a prospective cohort study

Dear editor,

Vaccines have proven to be safe, effective, and able to reduce the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and its variants, as well as abrogate the serious clinical consequences of coronavirus disease 2019 (COVID-19).[1,2] In this Journal, the report by Liu and co-workers evaluated the persistence of immunogenicity of seven COVID-19 vaccine, not including CoronaVac vaccine, at three months after third dose boosters, showing that the decay rates of humoral response vary among vaccines.[3] We undertook a study to evaluated the dynamic response and duration of anti-SARS-CoV-2 antibodies after a third dose of inactivated CoronaVac vaccine within 180 days and specifically assessed the decay of antibodies.

A prospective cohort study design was employed as we previously reported.[4] 41 participants received the three-dose CoronaVac vaccine (Fig. 1A) and provided blood donation at 8 serial time points within 180 days after the third dose. This study was approved by the Institutional Ethics Committee of Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University. All participants provided written informed consent. The neutralizing antibody, anti-RBD total antibody, anti-Spike IgG titers were serially determined to evaluate the immune response and duration. Mixed effects exponential and power law models were used to analyze antibody waning.

The seropositive rate of neutralizing antibody was 2.44% after the second dose (248 days). After the third dose, the seropositive rate reached 100% at two weeks, maintained for approximately 2 months and began to slowly decrease, dropping to 80.49% at 180 days (Fig. 1B). On the other hand, the level of antibody concentration rapidly increased from a base value of 5.03 IU/mL and peaked at 707.20 IU/mL at two weeks and then also began to slowly decline, remaining at 175.29 IU/mL at 180 days (Fig. 1C).

For the anti-RBD total antibody, the seropositive rate was 39.02% after the second dose, peaked at 100.00% one week after the third dose and was maintained within 180 days (Fig. 1B). The level of anti-RBD total antibody rapidly increased from a base value of 5.13 AU/mL to 177.27 AU/mL at one week after the third dose, peaked at 534.35 AU/mL within the three weeks, and then began to decline, dropping to 198.54 AU/mL at 180 days (Fig. 1D). The response for anti-Spike IgG after vaccination was similar to that for the anti-RBD total antibody (Fig. 1E).

To measure anti-SARS-CoV-2 antibody waning after vaccination, two mixed effects models were fitted. First, the neutralizing antibody, anti-RBD total antibody, and anti-Spike IgG levels declined over time, with half-lives of 81.14 days, 105.66 days, and 104.76 days within 180 days after the third dose, respectively, as estimated by an exponential decay model, which increased 2–4 fold compared with those after the second dose[5] and were longer than those within 3 months after the third dose in our previous study.[4] The power law model estimated half-lives for the neutralizing antibody of 293.88 days, anti-RBD total antibody of 468.98 days, and anti-Spike IgG of 467.28 days, which were longer than those estimated by the exponential decay model (Fig. 2A-C), indicating that the concentration of these antibodies may be starting to stabilize. Different antibodies were classified into two subgroups (younger participants ($\leq$33 years) and older participants ($>$33 years)) based on age. The results of two mixed effects models showed that younger participants had a higher likelihood of antibody persistence than older participants (Fig. 2D-F).

The findings of this study showed that 41 participants who received the third dose of the CoronaVac inactivated vaccine exhibited relatively good responses and durations of neutralizing antibody, anti-RBD total antibody and anti-Spike IgG and prolonged decay time, which were higher than expected.

Neutralizing antibody levels are highly predictive of immune protection.[6,7] Our results showed that the seropositive rate for neutralizing antibody was 80.49% at 180 days after the third dose vaccination, which was higher than that after the second dose that we had previously studied at this point in time.[5] The neutralizing antibody level declined over time which increased approximately 2-fold compared with that after the second dose[5] and was also longer than that within 3 months of the third dose in our previous study.[4] Our real-world data supported that the recall responses to boost doses in individuals with preexisting immunity primarily increased antibody levels and substantially altered antibody decay rates. More specifically, the importance of these observations is that neutralizing antibodies in vaccinees may persist, albeit with a relatively low rate of decay, and may act as the first line of defense against future encounters with the omicron variant or future variants evolved from omicron.

Although vaccination is key to preventing infections, vaccine responses are often found to be lower in elderly adults. Our results suggest that younger participants had a higher likelihood of neutralizing antibody persistence than older participants. The markedly reduced vaccine success in older adults has been attributed to adaptive immunosenescence.[8]

Limitations of this study include short follow-up time, small sample of persons, no detection of cellular responses and evaluated only homologous inactivated vaccinations and so on.

In conclusion, our results showed that the third vaccine dose dramatically increased antibody levels and prolonged the decay time, which were higher than we expected. Therefore, antibodies decay slowly in terms of immunity persistence such that there is no need to rush to deploy a fourth vaccination strategy, or a booster dose could be given to vulnerable groups first.

https://doi.org/10.1016/j.jinf.2022.06.003

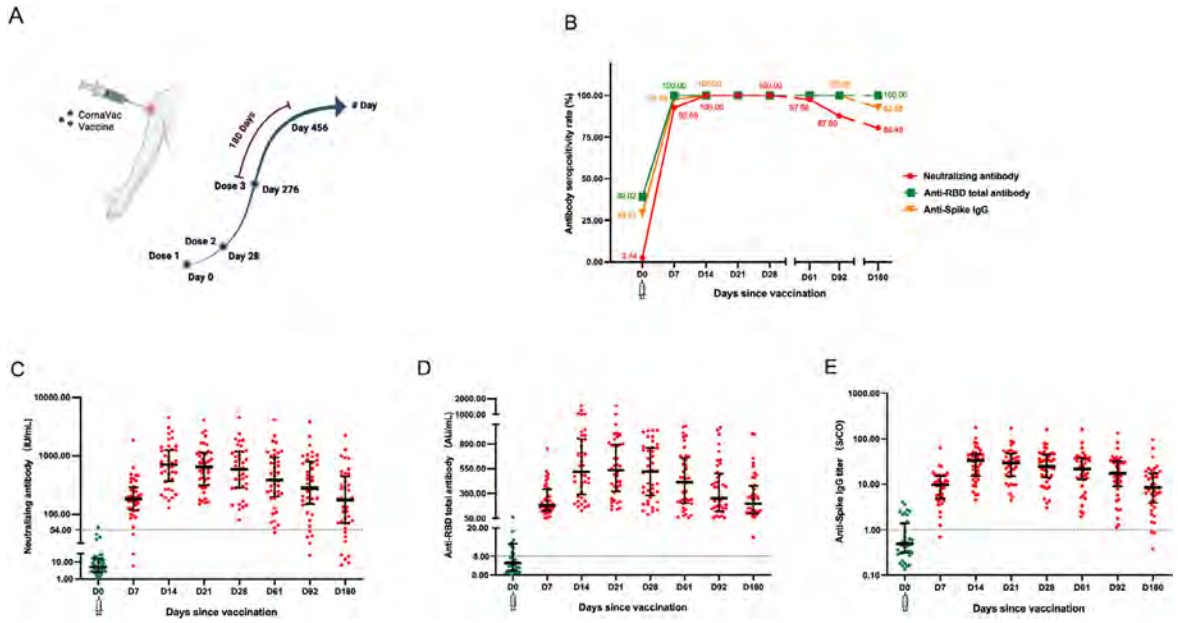


Fig. 1. Anti-SARS-CoV-2 antibody response after the third dose vaccination. A. Schedule of vaccination procedures. B. The seropositive rate changes of antibodies. C-E. The levels of neutralizing antibody (C), anti-RBD total antibody (D) and anti-Spike IgG (E) were measured at 8 serial time points. The antibody-positive judgement threshold is marked with a dotted line.

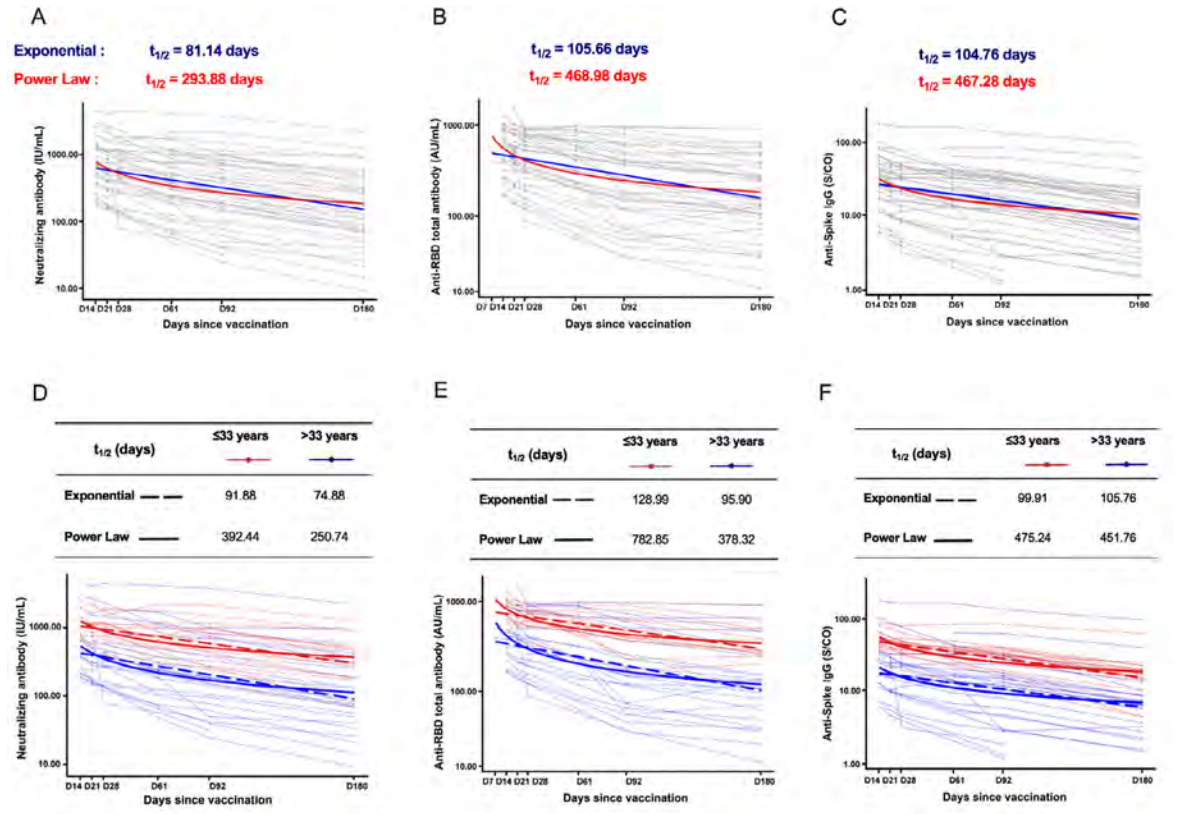


Fig. 2. The exponential and power law model of decay half-lives. A–C: A. Neutralizing antibody; B. Anti-RBD total antibody; C. Anti-Spike IgG. Antibody decay curves and half-lives estimated by an exponential decay model are shown in blue, and the decay curves and half-lives at day 120 estimated by a power law model are shown in red. D-F: D. Neutralizing antibody; E. Anti-RBD total antibody; F. Anti-Spike IgG. Antibody decay curves and half-lives estimated for younger participants (≤33 years) are shown in red, and older participants (>33 years) are shown in blue. Dotted lines represent exponential models, and solid lines represent power law model.

Funding

This work was supported by the National Natural Science Foundation of China [Grant Nos. 81973104, 81772260, and 81471967], the Key Projects for Province Science and Technology Program of Fujian Province, China [Grant No. 2018D0014], and the Natural Science Foundation of Fujian Province, China [Grant No. 2021J02055].

Declaration of Competing Interest

All the authors declare no competing interest in this work.

Acknowledgments

The authors thank all the contributors to this work. We thank Xiamen Boson Biotech Co, Ltd, Fujian, China for participant recruitment and sample collection.

References

1. Stasi C, Meoni B, Voller F, et al. SARS-CoV-2 Vaccination and the bridge between first and fourth dose: where are we? *Vaccines* 2022;**10**(3) (Basel)[published Online First: 2022/03/27]. doi:10.3390/vaccines10030444.
2. Su S, Du L, Jiang S. Learning from the past: development of safe and effective COVID-19 vaccines. *Nat Rev Microbiol* 2021;**19**(3):211–19 [published Online First: 2020/10/18]. doi:10.1038/s41579-020-00462-y.
3. Liu X, Munro APS, Feng S, et al. Persistence of immunogenicity after seven COVID-19 vaccines given as third dose boosters following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK: three month analyses of the COV-BOOST trial. *J Infect* 2022;**84**(6):795–813 [published Online First: 2022/04/12]. doi:10.1016/j.jinf.2022.04.018.
4. Liang XM, Xu QY, Jia ZJ, et al. A third dose of an inactivated vaccine dramatically increased the levels and decay times of anti-SARS-CoV-2 antibodies, but disappointingly declined again: a prospective, longitudinal, cohort study at 18 serial time points over 368 days. *Front Immunol* 2022:13. doi:10.3389/fimmu.2022.876037.
5. Xu QY, Xue JH, Xiao Y, et al. Response and duration of serum anti-SARS-CoV-2 antibodies after inactivated vaccination within 160 days. *Front Immunol* 2021;**12**:786554 [published Online First: 2022/01/11]. doi:10.3389/fimmu.2021.786554.
6. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021;**27**(7):1205–11 [published Online First: 2021/05/19]. doi:10.1038/s41591-021-01377-8.
7. Morales-Nunez JJ, Munoz-Valle JF, Meza-Lopez C, et al. Neutralizing antibodies titers and side effects in response to BNT162b2 vaccine in healthcare workers with and without prior SARS-CoV-2 infection. *Vaccines* 2021;**9**(7):1–13 (Basel)[published Online First: 2021/08/07]. doi:10.3390/vaccines9070742.
8. Müller L, Andrée M, Moskorz W, et al. Age-dependent immune response to the Biontech/Pfizer BNT162b2 Coronavirus disease 2019 vaccination. *Clin Infect Dis* 2021;**73**(11):2065–72 an official publication of the Infectious Diseases Society of America. doi:10.1093/cid/ciab381.

Qiu-Yan Xu[1], Qiu-Ling Li[1]
Centre of Clinical Laboratory, Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China
Institute of Infectious Disease, School of Medicine, Xiamen University, Xiamen, China

Zhi-Juan Jia, Meng-Juan Wu, Yan-Yun Liu
Xiamen Boson Biotech Co., Ltd, Xiamen, China

Li-Rong Lin*, Li-Li Liu*, Tian-Ci Yang*
Centre of Clinical Laboratory, Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China
Institute of Infectious Disease, School of Medicine, Xiamen University, Xiamen, China

*Corresponding authors at: Centre of Clinical Laboratory, Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen, China.
E-mail addresses: linlirong@xmu.edu.cn (L.-R. Lin), liulili@xmu.edu.cn (L.-L. Liu), yangtianci@xmu.edu.cn (T.-C. Yang)
[1] These authors contributed equally to this work.

7.4. Dose de reforço da CoronaVac aumenta em 325 vezes os anticorpos IgG contra a ômicron, mostra estudo

Um estudo realizado na província de Jiangsu, na China, mostrou que a dose de reforço da CoronaVac induz uma potente resposta imune contra as variantes delta e ômicron do vírus SARS-CoV-2, aumentando de 300 a 500 vezes o nível de anticorpos específicos produzidos contra essas cepas. O trabalho foi publicado na revista Emerging Microbes & Infections e conduzido por pesquisadores da Faculdade de Medicina da Universidade de Nanjing.

Os pesquisadores selecionaram 100 profissionais de saúde do Hospital da Universidade de Nanjing que receberam duas doses da CoronaVac em fevereiro de 2021. Após nove meses, quando a proteção contra o vírus SARS-CoV-2 reduz naturalmente, independente da vacina aplicada, uma terceira dose de CoronaVac foi administrada em 77 dos indivíduos. Amostras de soro foram coletadas em três momentos diferentes: antes da terceira dose, duas semanas depois e dois meses depois.

A dose de reforço da CoronaVac ativou a memória imunológica em todos os indivíduos. Antes da terceira dose, a titulação média dos anticorpos IgG anti-RBD (domínio de ligação ao receptor) era de 3.278 para o vírus original de Wuhan, 197 para a variante delta e 44 para a ômicron. Após o reforço, a titulação aumentou 17 vezes (para 56.760) contra a cepa original, 577 vezes (113.773) contra a delta e 325 vezes (14.336) contra a ômicron. Dois meses depois, os títulos permaneceram elevados, sendo de 82.666, 56.861 e 9.277, respectivamente.

"Nosso estudo destaca que a terceira dose da CoronaVac pode elevar significativamente as respostas de anticorpos que reconhecem as variantes delta e ômicron, em comparação com as duas doses da vacina. Além disso, a potência, amplitude e duração das respostas adaptativas melhoraram concomitantemente", apontam os autores.

Publicado em: 24/5/2022

Emerging Microbes & Infections

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/temi20

The third dose of CoronVac vaccination induces broad and potent adaptive immune responses that recognize SARS-CoV-2 Delta and Omicron variants

Yuxin Chen, Lin Chen, Shengxia Yin, Yue Tao, Liguo Zhu, Xin Tong, Minxin Mao, Ming Li, Yawen Wan, Jun Ni, Xiaoyun Ji, Xianchi Dong, Jie Li, Rui Huang, Ya Shen, Han Shen, Changjun Bao & Chao Wu

To cite this article: Yuxin Chen, Lin Chen, Shengxia Yin, Yue Tao, Liguo Zhu, Xin Tong, Minxin Mao, Ming Li, Yawen Wan, Jun Ni, Xiaoyun Ji, Xianchi Dong, Jie Li, Rui Huang, Ya Shen, Han Shen, Changjun Bao & Chao Wu (2022): The third dose of CoronVac vaccination induces broad and potent adaptive immune responses that recognize SARS-CoV-2 Delta and Omicron variants, Emerging Microbes & Infections, DOI: 10.1080/22221751.2022.2081614

To link to this article: https://doi.org/10.1080/22221751.2022.2081614

Publisher: Taylor & Francis & The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group, on behalf of Shanghai Shangyixun Cultural Communication Co., Ltd
Journal: *Emerging Microbes & Infections*
DOI: 10.1080/22221751.2022.2081614

The third dose of CoronVac vaccination induces broad and potent adaptive immune responses that recognize SARS-CoV-2 Delta and Omicron variants

Yuxin Chen[1,2*], Lin Chen[3*], Shengxia Yin[4,2*], Yue Tao[3], Liguo Zhu[5], Xin Tong[4,2], Minxin Mao[4], Ming Li[4], Yawen Wan[4], Jun Ni[1], Xiaoyun Ji[6,2], Xianchi Dong[7], Jie Li[4,2], Rui Huang[4,2], Ya Shen[5], Han Shen[1†], Changjun Bao[5†], Chao Wu[1,2†]

[1]Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China

[2]Institute of Viruses and Infectious Diseases, Nanjing University, Jiangsu, China

[3]Department of Laboratory Medicine, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, Nanjing, Jiangsu, China.

[4]Department of Infectious Diseases, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China

[5]Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, Jiangsu, China.

[6]State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing, Jiangsu, China

[7]Engineering Research Center of Protein and Peptide Medicine, Ministry of Education，Nanjing, Jiangsu, China

*These authors contributed equally to this work.

†**Correspondence to:**

Chao Wu, Department of Infectious Diseases, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, 210008, China, Email: dr.wu@nju.edu.cn; Changjun Bao, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, Jiangsu, 210008, China, Email:bao2000_cn@163.com; Han Shen, Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China, Email: shenhan10366@sina.com.

Keywords: COVID-19 Vaccine, booster, CoronaVac, neutralization, T cell responses

Abstract

The waning humoral immunity and emerging contagious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants resulted in the necessity of the booster vaccination of coronavirus disease 2019 (COVID-19). The inactivated vaccine, CoronaVac, is the most widely supplied COVID-19 vaccine globally. Whether the CoronaVac booster elicited adaptive responses that cross-recognize SARS-CoV-2 variants of concern (VoCs) among 77 healthy subjects receiving the third dose of CoronaVac was explored. After the boost, remarkable elevated spike-specific IgG and IgA responses, as well as boosted neutralization activities were observed, despite 3.0-fold and 5.9-fold reduced neutralization activities against Delta and Omicron strains compared to that of the ancestral strain. Furthermore, the booster dose induced potent B cells and memory B cells that cross-bound receptor binding domain (RBD) proteins derived from VoCs, while Delta and Omicron RBD-specific memory B cell recognitions were reduced by 2.7-fold and 4.2-fold compared to that of ancestral strain, respectively. Consistently, spike-specific circulating follicular helper T cells (cTfh) significantly increased and remained stable after the boost, with a predominant expansion towards cTfh17 subpopulations. Moreover, SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells peaked and sustained after the booster. Notably, $CD4^+$ and $CD8^+$ T cell recognition of VoC spike was largely preserved compared to the ancestral strain. Individuals without generating Delta or Omicron neutralization activities had comparable levels of $CD4^+$ and $CD8^+$ T cells responses as those with detectable neutralizing activities. Our study demonstrated that the CoronaVac booster induced broad and potent adaptive immune responses that could be effective in controlling SARS-CoV-2 Delta and Omicron variants.

Introduction

The high degree of waning humoral immunity and emerging contagious SARS-CoV-2 variants resulted in the occurrence of breakthrough infection [1] and the necessity of the booster vaccination of coronavirus disease 2019 (COVID-19). A recent real-world study in Israel suggested that the immunity against SARS-CoV-2 across all age groups was decreased a few months later after the second dose of immunization [2]. Meanwhile, SARS-CoV-2 Delta and Omicron variants have rapidly achieved widespread community transmission, accounting for most infections globally [3,4]. Particularly, Omicron harbors 30-40 mutations in spike protein including some substitutions which were previously confirmed to increase viral transmission and resist to neutralizing antibodies [5]. Due to the reduced efficacy of initial rollout of mass vaccination campaigns, the necessity of a third booster dose is constantly concerned [6].

There is still an ongoing debate that whether there is a need for booster vaccines owning to a lack of experiment evidence [7]. There is no consensus on the necessity of a third dose booster of COVID-19 vaccines in the whole population [8]. Nevertheless, WHO has recommended a third dose of inactive virus vaccine or a heterologous booster for people aged over 60 years who already have received the two-dose scheme [9], because of the pronounced decrease of neutralizing antibody (NAb) titers [10,11] and reduced effectiveness in older population [12]. A booster dose of the BNT162b2 vaccine could significantly lower the rates of confirmed COVID-19 and severe illness across age groups [13]. The third-dose vaccine developed by Pfizer-BioNTech [14], Oxford-AstraZeneca [15] and Sinovac [10] induced surging levels of infection-blocking ‘neutralizing’ antibodies. Among them, CoronaVac, a whole-virion inactivated vaccine produced by Sinovac, is the most widely offered COVID-19 vaccine globally [16]. Currently, a third booster dose of CoronaVac has been implemented among high-risk populations in China and other

countries. However, there is little research on the protective immune responses elicited by the CoronaVac boosters against variants of concern since the vaccine is being applied in countries deficient in research capacity and resources [10]. Meanwhile, the impact of the variant-associated mutations has been established for most variants regarding antibody reactivity [17-19], while much is less available for vaccine-induced T cell and B cell responses. Additionally, whether a third dose could effectively boost the waned humoral and cellular immunity remains unclear. Therefore, it is urgent to evaluate the cross-reactivity of SARS-CoV-2 humoral and cellular responses against the emerging variants elicited by CoronaVac booster, so as to provide essential data for the public health response to the COVID-19 pandemic.

We have reported the dynamic antibody, B cell and T cell responses following immunization of CoronaVac in a prospective cohort of 100 SARS-CoV-2 naïve healthcare professionals [20, 21]. It was revealed that 2-dose immunization effectively elicited spike-specific B cells, as well as SARS-CoV-2-specific $CD4^+$T cell and $CD8^+$ T cell responses. After 9 months, the third dose of CoronaVac booster was administered to 77 out of 100 healthcare professionals. In this study, their circulating antibody response, serum neutralization capacity, cellular responses including B cells, circulating T follicular helper cells (cTfh), as well as $CD4^+$ and $CD8^+$ T cell responses were closely monitored. The results demonstrated a necessity of a booster dose of CoronaVac, which can induce broad and potent adaptive immune responses effective in controlling SARS-CoV-2 Delta and Omicron variants.

Materials and Methods

Study cohort and sample collection

Previously, we conducted in a prospective, observational study (NCT04729374) in Nanjing Drum Tower Hospital, Jiangsu, China. All participants were tested negative for SARS-CoV-2 infection at screening and provided written informed consent. The clinical trial protocol was approved by hospital ethics committee (2021-034-01). After 9 months after two-dose, the third dose of CoronaVac was administered to 77 healthcare professionals during the period from Nov 8th to Nov 14th, 2021. Serum samples for detailed immunological assessments were taken at three different time points, including before the third dose (T0), day 14 post the third dose (T1), and day 56 post the third dose (T2) **(Figure 1).** The antibody titer and serum neutralization activity from this cohort were also compared to that of a breakthrough infection cohort, consisting of 10 subjects with Delta breakthrough infection after the two-dose vaccine from CoronaVac. Sera from the breakthrough cohort were obtained between day 13-18 post disease onset.

Peripheral blood sample processing

Blood samples were collected via phlebotomy in acid citrate dextrose serum separator tubes, or ethylenediaminetetraacetic acid (EDTA) anticoagulated tubes. Peripheral blood mononuclear cells (PBMCs) were isolated from blood collected in EDTA tubes by lymphocyte separation medium density gradients (Stemcell Technologies, Vancouver, Canada) and resuspended in PRMI 1640 medium supplemented with 10% fetal calf serum (FCS), 1% penicillin/streptomycin and 1.5% HEPES buffer (Thermo Fisher Scientific, MA, USA) for stimulation assays or stored at - 135°C until used.

Proteins and peptides

Pools of 15-mer peptides overlapping by 11 amino acid and together spanning the entire sequence of SARS-CoV-2 spike glycoprotein (S) from ancestral, Alpha (B.1.1.7) and Delta (B.1.617.2) variants, wild-type virus open reading frame 3a, ORF7 and ORF8 (ORF3a/7/8), membrane protein (M), and envelope small membrane (E) were synthesized (Genscript, Jiangsu, China) and used for *ex vivo* stimulation of PBMCs.

The ectodomain of ancestral SARS-CoV-2 spike (GenBank: MN908947.3) was expressed as previously described [23]. The prefusion Omicron (B.1.1.529/21K) spike ectodomain (GenBank: OL672836.1, residues 1-1205) was cloned into vector pcDNA3.1 (Thermo Fisher Scientific, MA,USA) with proline substitutions at residues 983 and 984, a “GSAS” instead of “RRAR” at the furin cleavage site (residues 679-682), with a C-terminal T4 fibritin trimerization motif, an HRV-3C protease cleavage site, a Twin-Strep-tag, and an 8×His-tag according to Jason S. McLellan’s research [24]. The protein was purified from FreeStyle 293-F cells (Thermo Fisher Scientific, MA, USA) using affinity chromatography followed by size exclusion chromatography, detailed as described previously [23].

Measurement of SARS-CoV-2 spike and RBD-specific IgG and IgA titer

Antigen-specific serological antibodies against SARS-CoV-2 were determined by enzyme-linked immunosorbent assay (ELISA) [20, 21]. Briefly, 96-well plates were coated with 500 ng/mL of each recombinant viral antigen overnight. The plates were incubated with serum samples in a dilution of 1:200, followed by incubation with either anti-human IgG conjugated with HRP (ab6759, Abcam, Cambridge, England) or anti-human IgA conjugated with HRP (ab97215, Abcam, Cambridge, England). Subsequently, the plates were incubated with TMB substrate for 1

hour and the reaction stopped with 1M H_2SO_4. Optical density (OD) value at 450 nm was measured. The cut-off value was determined as the average of OD values plus 2 standard deviations (SD) from 45 archived healthy individuals from the year of 2019 as the unexposed donors. Antibody endpoint titer was determined by the highest dilution of serum which gives an OD value higher than cut off value of the healthy control group at the same dilution.

Pseudovirus Neutralization Assay

Pseudovirus neutralization assay was performed as previously described to evaluate the serum neutralization capability that highly correlated with authentic neutralization assay [20, 22]. Briefly, the lentivirus-based SARS-CoV-2 pseudoviruses were provided by Vazyme Biotech Co.,Ltd (Nanjing, China), which bear the spike protein derived from the D614G variant, the Delta variant (B.1.617.2), and the Omicron variant (B.1.1.529). SARS-CoV-2 pseudovirus was produced by co-transfection of a HIV-1 NL4-3 luciferase reporter vector that contains defective Nef, Env and Vpr (pNL4-3.luc.RE) and a pcDNA 3.1 expression plasmid (Invitrogen, Thermo Fisher Scientific, MA,USA) encoding respective spike protein in 293T cells. After 48 hours, cell supernatants containing pseudoviruses were collected, filtered, and stored at -70˚C until use. The 50% tissue culture infectious dose ($TCID_{50}$) of SARS-CoV-2 pseudovirus was measured by luciferase assay in relative light units (RLUs). To determine the neutralization activity of vaccinee serum, three-fold serial dilution starting from 1:30 were performed for heat-inactivated serum samples in duplicated before adding $1x10^3$ $TCID_{50}$ pseudoviruses per well for 1 hour, together with the virus control and cell control wells. The mixture was added to 2 x 10^4 HEK293T-ACE2 cells (Cat# DD1401-01, Vazyme, Nanjing, China) per well and incubated for 48 hours in 5% CO_2 environment at 37˚C. The luminescence was measured using Bio-lite Luciferase assay system

(Cat# DD1201-01, Vazyme, Nanjing, China) and detected for RLUs using Spark multimode microplate reader (Tecan, Männedorf, Switzerland). The titer of neutralization antibody (ID_{50}) was defined as the reciprocal serum dilution at which the relative light units (RLUs) were reduced by 50% compared to the virus control wells after background RLUs in the control groups with cells only was subtracted. Data for pseudovirus neutralization titers for the D614G variant after 2-dose CoronaVac were reported previously [20].

Antigen-Specific Measurement of Cellular analysis

Antigen-specific measurement of cellular analysis was performed as previously described [21]. For RBD-specific B cell analysis, PBMC samples were incubated with 100 ng fluorescence APC or PE labeled RBD protein at 4°C for one hour to ensure maximal staining quality followed by surface staining with antibodies at 4°C for 30 min. The following antibodies for phenotypic B cell surface markers were used, including anti-CD19-BV421 (Clone HIB19, 1:50), anti-CD27-BV655 (Clone MT-271, 1:50), anti-CD45-PE-cy7 (Clone HI30, 1:50), anti-CD3-Percp-cy5.5 (Clone OKT3, 1:50), anti-CD14-Percp-cy5.5 (Clone rmC5-3, 1:50), anti-CD16-Percp-cy5.5 (Clone B73.1,1:50), anti-CD56-Percp-cy5.5 (Clone B159, 1:50), anti-IgD-FITC (Clone IA6-2,1:50). Fixable viability Dye eFluor 780 staining was used to exclude dead cells. The above antibodies were purchased from BD Biosciences (San Diego, USA). The frequency of circulating RBD-specific B cells was expressed as the percentage of total B cells ($CD19^+CD20^+CD3^-CD14^-CD16^-CD56^-$LIVE/$DEAD^-$lymphocytes). The frequency of antigen-specific RBD-specific memory B cells were expressed as percentage of total memory B cells ($CD19^+CD20^+CD27^+CD3^-CD14^-CD16^-CD56^-$LIVE/$DEAD^-$lymphocytes). Gating strategy for B cell analysis is shown in **Supplementary figure 1.**

To measure antigen specific circulating $CD4^+$ T cells, $CD8^+$ T cells and cTfh cells, activation-induced marker (AIM) assay was performed. Activation-induced marker (AIM) assay is a recently developed as a cytokine-independent method, capable of detecting early responding antigen-specific $CD4^+$T cells, $CD8^+$ T cells, and cTfh cells [25-27]. 1 x 10^6 fresh PBMCs were suspended in Roswell Park Memorial Institute (RPMI) medium and stimulated with peptides pools at a final concentration of 1µg/mL overnight. A stimulation with an equimolar amount of dimethylsulfoxide (DMSO) was performed as negative control, and PMA/Ionomycin as positive control. Following stimulation, cells were stained in flow buffer (DPBS, Gibco, NY, USA) supplemented with 2% FCS for 20 minutes at 4°C for viability. For $CD4^+$T cell and $CD8^+$ T cell analysis, the following antibodies were included for phenotypic lymphocyte surface markers: anti-CD3-PerCP-cy5.5 (Clone OKT3, 1:25), anti-CD4-Qdot655 (Clone RPA-T4, 1:50), anti-CD8-BV421 (Clone SK1, 1:25), anti-OX40-FITC (Clone L106, 1:50), anti-4-1BB-PE (Clone C65-485, 1:50), anti-CD45-PE-cy7 (Clone HI30, 1:50), anti-CD69-APC (Clone FN50, 1:25). Fixable viability Dye eFluor 780 staining was used to exclude dead cells. Gating strategy for T cells is shown in **Supplementary figure S2**. AIM^+CD4^+ T cells were defined based on dual expression of 4-1BB and OX40, and AIM^+CD8^+ T cells were identified based on dual expression of 4-1BB and CD69. The fraction of $CD4^+$ or $CD8^+$ T cells responsive to 5 overlapping peptide pools covering the ancesral spike glycoprotein, nucleoprotein (N), membrane protein (M), envelope small membrane protein (E), ORF3a/7/8 were then added as a combined sum of SARS-CoV-2 specific $CD4^+$ or $CD8^+$ T cells. For Tfh cell analysis, the following antibodies were used for phenotypic lymphocyte surface markers: anti-CD3-PerCP-cy5.5 (Clone OKT3, 1:25), anti-CD4-Qdot655 (Clone RPA-T4, 1:50), anti-OX40-FITC (Clone L106, 1:50), anti-4-1BB-PE (Clone C65-485, 1:50), anti-CD45-BV421

(Clone HI30, 1:50), anti-CXCR5-CF594 (Clone RF8B2, 1:50), anti-CCR6-APC (Clone 11A9, 1:50), anti-CXCR3-PE-cy7 (Clone 1C6, 1:50). Fixable viability Dye eFluor 780 staining was used to exclude dead cells. Spike-specific cTfh cells were gated as $OX40^{+}4\text{-}1BB^{+}CXCR5^{+}CD4^{+}$T cells and were further divided into cTfh1($CXCR3^{+}CCR6^{-}$), cTfh2($CXCR3^{-}CCR6^{-}$), cTfh17($CXCR3^{-}CCR6^{+}$) and cTfh1-17($CXCR3^{+}CCR6^{+}$). Gating strategy for cTfh cells is shown in **Supplementary figure S3.** Antigen-specific $CD4^{+}$ T cell, $CD8^{+}$ T cell and cTfh cell responses determined by AIM assays were calculated as background (DMSO) subtracted data. The above antibodies were purchased from BD Biosciences (San Diego, USA). The lower limit of detection (LOD) for cellular analysis was calculated using the median two-fold standard deviation of all negative control samples from the unexposed donors. Staining samples were analyzed by a fluorescence-activated cell sorter (FACS) Aria™ III Cell Sorter instrument (BD Biosciences) using FlowJo software (version 10).

Statistical analysis

Binding antibody titers or neutralization titers were expressed as geometric mean titers (GMTs). The mean (standard deviation (SD)) or median (interquartile range (IQR)) was used to present the continuous variables. Categorical variables were described as the counts and percentages. Wilcoxon matched-pairs signed rank was used for comparison between timepoints and between SARS-CoV-2 variants. Unpaired wilcoxon test for comparison between groups. Correlation between 2 continuous variables was analyzed using the spearman correlation analysis. $p<0.05$ was considered as statistically significant. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p< 0.001$, **** indicates $p< 0.0001$, and ns indicates no significant difference. SPSS software program version 22.0 (Chicago, IL, USA) was used for data analysis.

Results

Cohort design

A prospective observational study to follow vaccine-induced immune response previously characterized the longitudinal magnitude of antibody response, as well as $CD4^+$ and $CD8^+$ T cells in a prospective observational cohort that received two-dose of CoronaVac [20-21]. Seventy-seven healthy individuals from this original cohort received the third dose of CoronaVac 9 months after the priming two-dose vaccination **(Figure 1 and Table 1).** Specifically, sampling at pre-boost (T0), 2 weeks (T1) and 8 weeks (T2) after the third immunization of CoronaVac enabled the dynamic immune analysis of SARS-CoV-2 specific humoral responses, B cells, $CD4^+$ T cells, $CD8^+$ T cells and cTfh cells. Paired serum and peripheral blood mononuclear cell (PBMC) samples were collected from all individuals, allowing detailed analyses of both serological and cellular immune responses to SARS-CoV-2 antigens derived from different variants. Furthermore, a control donor group was set for humoral responses, in which 10 subjects with Delta breakthrough infection were prior fully vaccinated with CoronaVac. In this way, the dynamics of re-activating pre-existing immunity elicited by SARS-CoV-2 inactivated vaccines were investigated.

Boosted Antibody responses to SARS-CoV-2 Ancestral, Delta and Omicron spike antigens

First, the pre- and post-boost IgG serum titers against the ancestral (Wuhan-1), Delta (B.1.617.2) and Omicron (B.1.1.529) RBD and spike proteins were measured in our vaccine cohort **(Figure 2)**. A booster dose of CoronaVac elicited a strong recall response in all individuals, with increased anti-RBD and anti-spike IgG and IgA titers compared with pre-boost titers. Baseline sera exhibited an anti-ancestral RBD-specific binding immunoglobulin G (IgG) geometric median titer (GMT)

of 3,278 (95% CI, 1,953-5,504), while GMT specific to Delta RBD was 197 (84-460) and omicron RBD was 44 (16-120), respectively. After the booster dose, anti-ancestral RBD-specific binding IgG was increased to a GMT of 56,760 (38,284-84,151); the GMTs for anti-Delta RBD IgG and anti-Omicron RBD IgG were 113,773 (94925,136363) and 14,336 (11025,18641), respectively. The IgG titers stayed stable for 2 months after the booster and the IgG GMTs specific to ancestral RBD, Delta RBD and Omicron RBD were 82,666 (57308, 119244), 56861 (45630, 70856) and 9277 (5903, 14581), respectively **(Figure 2A).** Notably, anti-Delta RBD specific IgG titers at T1 and T2 timepoint were 1.1-fold and 4.0-fold lower compared to that specific to ancestral RBD, respectively **(Figure 2E)**. The booster recipients presented 9.2-fold and 13.8-fold decreased anti-Omicron RBD specific IgG titer compared to those of anti-ancestral RBD protein after 2 weeks and 8 weeks, respectively. Meanwhile, a low level of anti-RBD IgA responses was detected. The anti-ancestral RBD IgA at baseline possessed a GMT of 6.7 (3.0-15.0), elevated to 1009 (562.8-1808) after the booster, and then dropped to 376 (173.3-814.1) 2 month after the booster **(Figure 2B and 2F)**. Consistently, spike-specific IgG and IgA also followed a similar trend as RBD-specific IgG and IgA responses **(Figure 2C-2D and 2G-2F)**. The breakthrough cohort, as a crucial control, demonstrated a comparable level of IgG responses specific to ancestral RBD (p=0.18) and omicron RBD (p=0.07), but significantly higher level of IgG responses specific to Delta RBD (6.48-fold, p=0.02) compared to those of 3-dose recipients at T1 timepoint, respectively. Similarly, anti-Omicron RBD IgG titer was 7.8-fold lower than that of anti-ancestral RBD in the breakthrough cohort **(Figure 2E).** Besides, the breakthrough cohort exhibited a significantly higher level of IgA titer specific to ancestral RBD protein and Delta RBD protein but a comparable level of Omicron RBD-specific IgA responses, compared to the booster vaccine cohort at the T1 timepoint. Our data suggested that the booster dose can not only increase the magnitude of IgG

and IgA responses but also broaden the antibody responses specific to spike protein derived from emerging viral variants.

Improved potent and broad neutralization against emerging SARS-CoV-2 variants

Our previous study reported that serum from 2-dose CoronaVac recipients in our cohort can effectively neutralize D614G and Alpha variants [20]. In this study, the neutralization titers against D614G, Delta, and Omicron variants were analyzed for serum collected at 2 weeks post 2-dose and 3-dose CoronaVac immunization to determine whether a third CoronaVac dose could increase the potency and breadth of serum neutralization activities **(Figure 3A).** Most (98.7%, 76/77) 3-dose recipients can neutralize against D614G with a GMT of 172.9 (141.8-210.9) compared to a GMT of 42.3 (34.1-52.5) after 2 doses. Meanwhile, the breakthrough infection cohort presented a surging neutralizing GMT of 3581.0 (1601.0-8012.0). Additionally, 89.6% (69/77) of booster sera neutralized against Delta strain (GMT 64.8, 53.4-78.5) with a 5.0-fold increase compared to serum samples from 2-dose vaccinees. However, the breakthrough infection resulted in a GMT of 664 (373.7-1181.0) against Delta strain. Booster recipients and breakthrough cohort demonstrated 3.0-fold and 6.7-fold less neutralization susceptible than D614G, respectively **(Figure 3B)**. Meanwhile, 43 (55.8%) of subjects revealed neutralizing activities against Omicron strain after the boosting dose, with a 3.6-fold increased GMT from 16.1 (15.3-16.9) to 33.8 (27.7-41.5). All subjects with breakthrough infection possessed a neutralization capability with a GMT of 289.5 (143.5-584.1) for Omicron. Booster recipients and breakthrough cohort had 5.9-fold and 15.6-fold lower neutralization potency against the Omicron strain, respectively, compared to the ancestral strain. Our data suggested that a booster vaccine not only strongly enhance overall neutralizing potency

against SARS-CoV-2 but also strengthen the broad recognition for D614G strain, Delta and Omicron strains.

Strong correlations were observed between the magnitudes of IgG or IgA responses specific to ancestral RBD and that of antibody responses specific to VoC RBD **(Figure 3C)**. Consistently, serum neutralization titers for D614G, Delta, and Omicron strains were highly correlated with each other, respectively **(Figure 3D)**. Delta neutralization titer was strongly correlated with anti-Delta spike IgG responses ($r=0.57$, $p<0.0001$). Omicron neutralization titer was moderately related to anti-Omicron spike IgG ($r=0.44$, $p<0.001$) and anti-Delta spike IgG ($r=0.35$, $p<0.0001$). Besides, the possible factors that might affect the neutralization activities were explored due to heterogeneous neutralization potency observed in our cohort. Our work [21] and other studies [28] have demonstrated age-dependent neutralization activities for SARS-Cov-2 ancestral and emerging VoCs among CoronaVac or mRNA recipients. Nevertheless, 3-dose recipients under the age of 40 and over the age of 40 generated comparable magnitudes of neutralization activities **(Figure 3E).** Furthermore, whether pre-existing neutralization activities might affect the boosted humoral immunity was assessed. The serum neutralization activities on week 2 after 2 doses of CoronaVac in this cohort were previously reported [20]. Our cohort was further divided into two groups: individuals with prior low neutralization activities (serum $ID_{50} < 50$) and individuals with prior high neutralization activities (serum $ID_{50} \geq 50$). Our data implied that previous low neutralizers still had reduced serum neutralization activities compared to that of high neutralizers, even after the CoronaVac booster. Nonetheless, there was no correlation between neutralization titer after 2 doses and neutralization titer after 3 doses **(Figure 3F).**

B cell responses to SARS-CoV-2 ancestral, Delta and Omicron RBD protein

Two-dose CoronaVac induced potent RBD-specific B cells and memory B cells [21]. Here, the percentages of circulating RBD-specific B cells and its memory B cell subsets in booster recipients were measured**.** At baseline, RBD-specific B cells were still detectable in 86% of individuals. RBD-specific B cells were significantly expanded by 2.65-fold after the booster of CoronaVac, from 0.025% [0.007-0.042] to 0.049% [0.025, 0.067] ($p<0.005$), and slightly declined by 2.26-fold to an average frequency of 0.039% [0.010, 0.055] at T2 timepoint **(Figure 4A)**. RBD-specific memory B cells (MBCs) represented a large fraction of the neutralizing MBC pool against SARS-CoV-2, expanding substantially by 2.46-fold after the additional dose of CoronaVac (0.007% [0.005-0.009] versus 0.018% [0.015-0.021], $p<0.005$), and slightly decreased by 1.7-fold (0.012 [0.009-0.015]) 2-month after the booster dose **(Figure 4B).** Additionally, the recognition breadths of RBD-specific B cells and their memory subsets at T2 timepoint were also analyzed. The 3-dose CoronaVac recipients had 0.022% (0.017, 0.026%) and 0.020% (0.0015, 0.025%) of circulating B cells recognizing Delta and Omicron RBD, respectively, corresponding to 2.3-fold and 2.8-fold reduction in the percentage of VoC RBD-specific B cells compared to that of ancestral RBD-specific B cells **(Figure 4C).** A third dose also resulted in detectable RBD-specific memory B cells recognizing Delta (0.008% [0.006, 0.010%], by a factor of 2.7) and Omicron variant (0.007% [0.005, 0.009%], by a factor of 4.2), respectively, compared to ancestral RBD-specific B cells **(Figure 4D).** Therefore, RBD-specific B cell recognition was also partially affected by emerging VoC strains.

SARS-CoV-2 spike specific circulating T follicular helper cell responses

Tfh cell responses are necessary to form and sustain germinal center (GC) reactions, critical to develop long-lasting, high-affinity antibody responses [29, 30]. Besides, circulating Tfh (cTfh) cells in the peripheral blood resemble GC Tfh cells and serve as a counterpart to GC Tfh cells to support antibody secretion due to a similar phenotype [31]. Here we sought to track and depict spike-specific cTfh responses over time. Compared to minimal level of detectable spike-specific cTfh cells at baseline (0.001%), they peaked in peripheral blood 14 days at 1.165% of frequency after CoronaVac boost and remained at a median frequency of 0.81% after 2 months of the third dose immunization **(Figure 5A).** Additionally, they were compared with cTfh responses specific to VoC spike by testing spike peptides corresponding to the viral sequences of the Alpha and Delta strains. Interestingly, a small fraction of responders exhibited a loss of cTfh cell recognition of Delta (7/90; 7.8%) or Omicron (12/90; 13.3%) at T1 timepoint. Slight reductions of cTfh responses to Alpha spike (16%) and Delta spike (9%) were observed. At T2 timepoint, cTfh cell responses specific to Alpha, Delta and Omicron spike remained stable at the frequencies of 0.47%, 0.70% and 0.53%, respectively. The frequencies of Alpha or Delta spike-specific cTfh cells were significantly lower than those of ancestral spike at T1 timepoint (2.5-fold and 3.3-fold, respectively) **(Figure 5A and 5B)**, but not T2 timepoint **(Figure 5A).** The frequency of Omicron spike-specific cTfh cells was highly correlated with the frequency of ancestral spike-specific cTfh cells ($r=0.53$, $p<0.0001$), Alpha spike-specific cTfh cells ($r=0.50$, $p<0.0001$) and Delta spike-specific cTfh cells ($r=0.51$, $p<0.0001$) **(Figure 5C).**

With the purpose of extending these findings, the phenotypic characteristics of SARS-CoV-2 spike-specific cTfh cells were investigated using CXCR3 and CCR6 chemokine receptor markers **(Figure 5D)**. CXCR3 and CCR6 were adopted to identify the distinct B cell helper functions,

including cTfh1 ($CXCR3^+CCR6^-$), cTfh2 ($CXCR3^-CCR6^-$), cTfh1-17 ($CXCR3^+CCR6^-$), and cTfh17 ($CXCR3^-CCR6^+$) subsets [31-32]. cTfh2 and cTfh17 cells can induce B cell differentiation and antibody secretion and regulate immunoglobulin (Ig) isotype switching; cTfh1 cells are commonly considered not to be an effective helper for B cells. At T0 baseline, the phenotypic analysis of total cTfh cells from booster recipients revealed that cTfh1, cTfh2, cTfh17, and cTfh1-17 subsets occupied 19.1% (12.81% to 25.1%), 44.8% (37.2%-53.0%), 13.8% (8.0% -17.1%), and 22.3% (9.3%-31.6%), respectively. Interestingly, boosters exhibited the skewed distribution of cTfh cells toward the cTfh17 phenotype after 2 weeks (32.7%, 28.1%-37.5%) and after 8 weeks (44.1%, 39.2-48.6%), whereas cTfh2 subsets remained at a similar proportion. Concurrently, cTfh1 subsets gradually declined to 15.3% (10.1%-18.0%) at T1 and 6.9% (5.2%-8.6%) at T2 timepoint, while cTfh1-17 subsets decreased to 11.7% (8.5-14.1%) at T1 and 9.9% (7.7-11.9%) at T2**.** Thus, the CoronaVac booster can efficiently expand cTfh17 subsets, contributing to efficient secretion of IgG and IgA [31].

SARS-CoV-2 specific $CD4^+$ and $CD8^+$ T cell responses to different SARS-CoV-2 variants in booster recipients

Beyond antibodies and memory B cells, T cells can contribute to protection upon re-exposure to the virus. Activation-induced marker (AIM) assay was used to measure SARS-CoV-2 $CD4^+$ and $CD8^+$ T cell responses with the overlapping peptide pools from the ancestral strain. Firstly, SARS-CoV-2 specific $CD4^+$ T cells were detected in 66.7% of individuals with an average frequency of 0.198% 9 months after two vaccine doses **(Figure 6A).** A significant elevation to 1.54% for SARS-CoV-2-specific $CD4^+$ T cell responses was observed, and such positive responses were detected in 98.8% of individuals after the booster dose. SARS-CoV-2 specific $CD4^+$ T cell responses slightly

decreased to 0.72% ($p<0.0001$) and were detectable in 95.2% of participants at T2 timepoint. Similarly, spike-specific $CD4^+$ T cell responses followed a similar trend except that they remained at a high level 2 months after the booster dose**.** Since T cell responses were less affected by VoCs than humoral immune responses [33,34], the cross-reactivity of $CD4^+$ T cell responses to spike proteins derived different SARS-CoV-2 variants in our cohort was tested. $CD4^+$ T cell responses to Alpha and Delta spike were reduced compared to that in ancestral spike at T1 timepoint, as demonstrated by 3.9-fold and 3.2-fold reduction **(Figure 6C).** Meanwhile, a smaller effect of Alpha, Delta and Omicron mutations on $CD4^+$ T cell responses was observed at T2 timepoint, compared to that of T1 timepoint, as revealed by 1.2-fold~1.6-fold change**.** Similar results were observed for $CD8^+$ T cell responses at similar frequencies**.** At baseline, SARS-CoV-2 specific to $CD8^+$ T cells was detected in 61.9% of individuals with a frequency of 0.196% **(Figure 6B).** It significantly elevated to 1.45% in 98.8% of individuals 2 weeks after booster at T1 timepoint, and gradually declined to 0.97% while remaining positive in 95.2% of subjects at T2 timepoint. The magnitude of the SARS-CoV-2 specific $CD8^+$ T cell responses against ancestral strain, Alpha variant and Delta variant were significantly boosted from the baseline of 0.001% to 0.49%, 0.30% and 0.31% after the third dose, respectively, and maintained at a similar magnitude at T2 timepoint. The recognition of $CD8^+$ T cells to Alpha and Delta spike was decreased by 1.7-fold and 2.1-fold, respectively, at T1 timepoint, and the recognition of $CD8^+$ T cells to Alpha, Delta and Omicron spike was slightly decreased by 1.5-fold, 2.0-fold and 2.1-fold, respectively, at T2 timepoint **(Figure 6D).** Additionally, both Delta spike-specific $CD4^+$ and $CD8^+$ T cell responses were compared between neutralizing antibody (NAb) responders and NAb non-responders in the booster cohort. Regardless of NAb responses against Delta or Omicron strain, there are comparable levels of $CD4^+$ and $CD8^+$ T cell responses specific to Delta spike or Omicron spike **(Figure 6E).**

These results revealed that $CD4^+$ and $CD8^+$ T cell recognition of VoC spike is largely preserved compared to the ancestral strain.

Discussions

Substantial efforts have been made to speed up booster vaccination campaigns given the rapid spread of omicron worldwide. Our understanding of the vaccine-elicited immunological features associated with the main VoCs is the key to informing health policies including boosting vaccination schedules. This also contributed to the development of potential variant-specific or pan-coronavirus vaccines.

The dynamic humoral and cellular responses in a cohort of CoronaVac booster to emerging SARS-CoV-2 variants including ancestral, Delta and Omicron strains were analyzed in this study. Consistent with previous reports [34, 35], a substantial improved humoral immunity after CoronaVac boost or breakthrough infection was observed in our study. The third dose of CoronaVac significantly increased not only IgG responses but also IgA responses specific to spike protein. Notably, secretory IgA might play an essential role in protecting the mucosal surface against SARS-CoV-2 [36]. The booster of CoronaVac enhanced both the seroconversion rate of Delta and Omicron neutralization and the neutralizing potency, highlighting the necessity of a third dose of CoronaVac. In line with a previous report [37], breakthrough infection after two-dose vaccination of COVID-19 inactivated virus vaccine resulted in a natural booster to humoral immunity against SARS-CoV-2. Moreover, breakthrough infection induced significantly higher neutralization titer against SARS-CoV-2 variants compared to the boosting of CoronaVac, though

there are comparable levels of binding antibody titer specific to these VoC antigens. This might be caused by distinct routes of antigen exposure between vaccination and nature infection.

There are still knowledge gaps in our understanding of VoCs regarding to the vaccine-elicited cellular immune activity. The role of cellular immune responses and activated T-B cells stimulated by the antigen might be more imperative than circulating antibodies. Circulating spike-specific B cells may have crucial contributions to protective immunity by making anamnestic neutralizing antibody responses after infection. The continued maturation of B cell responses over time would assist in adapting SARS-CoV-2 immunity to VoCs [38]. Reduced B cell binding of RBD protein from variants was observed in all cases, while the reduction was less than 5-fold for Delta spike and Omicron RBD protein. This demonstrated a partial retained B cell recognition of variants, consistent with the observations that Omicron neutralizing antibody titers rapidly increased after the third immunization or breakthrough infection but were generally low among individuals with two-dose of CoronaVac.

The direct evaluation of key Tfh immunological events in lymphoid tissues after immunization is challenging in humans, making surrogate biomarkers such as cTfh cells in the blood especially informative [39]. In COVID-19 recovered individuals, spike-specific cTfh differentiated subjects were associated with potent neutralizing responses [40]. Robust Tfh cells were detected in paired blood and lymph node specimens from SARS-CoV-2 mRNA vaccinated individuals [41], which persisted at a nearly constant frequency for at least six months. Similarly, our study revealed that the third dose of CoronaVac induced robust and persistent spike-specific cTfh cell responses, which were correlated with the vaccine-induced RBD-specific B cells and serum neutralization

potency. We firstly verified that the expanded cTfh cell responses induced by CoronaVac booster exhibited a clear phonotypic bias toward a pro-inflammatory Tfh17 subset, previously reported for other viral glycoproteins [42]. Additionally, the magnitude of spike-specific cTfh cells remained unchanged and was less sensitive to mutations within VoC spikes, ranging from a 1.8-fold to 3.3-fold decrease. Therefore, the booster dose-induced cTfh cells can rapidly expand and further facilitate memory B cells to evolve, providing effective humoral responses upon virus re-exposure.

Distinct from B cell and cTfh cell recognition, our data suggested that the third dose of CoronaVac elicited broadly cross-reactive cellular immunity against SARS-CoV-2 variants including Delta and Omicron. The magnitude of Omicron cross-reactive T cells was comparable to that of Alpha and Delta variants, though the Omicron spike has greater number of mutations. Our observation was also in good agreement with previous studies that the effect of variant mutations on global $CD4^+$ and $CD8^+$ T cell responses was negligible [43-45] due to highly conserved $CD4^+$ and $CD8^+$ T cells epitopes within the viral variants [46]. The mutations derived from Delta and Omicron extended a limited impact on T cell responses, suggesting that vaccination or prior infection could provide substantial protection from severe disease. Indeed, these well-preserved T cell immunity to Delta or Omicron acquired through vaccination or infection might contribute to protection from severe COVID-19, consistent to lower risk of hospitalization and reduced disease severity observed in recent Omicron wave from South Africa [47].

Currently, the correlate of protection for vaccine against SARS-CoV-2 remains elusive, though the humoral and cellular responses induced by vaccines are well characterized. Neutralizing antibodies could serve as a correlate of protection for vaccines against SARS-CoV-2, while

antibody testing might lead to misperception and misunderstanding of vaccine effectiveness among the general population. Our study revealed that those NAb non-responders also had a similar magnitude of T cell responses, compared to NAb responders, suggesting that those without neutralizing antibody responses also benefited from the vaccine owing to robust and persistent T cell responses acquired by immunization.

This study has several limitations. First, this study was deficient in the data on the long-term follow-up of humoral and cellular responses after the third boost of CoronaVac. Follow-up studies should be conducted to monitor the duration and persistence of adaptive immune response. Additionally, we did not characterize the phenotypic memory differentiation for SARS-CoV-2 specific $CD4^+$ and $CD8^+$ T cell responses, nor test SARS-CoV-2-specific T cells responses in breakthrough infection cohort. Thus, longitudinal T cells responses elicited by either booster vaccination or breakthrough infection should be compared in future studies.

To summarize, our study highlighted that a booster dose of CoronaVac can provide give a significantly larger boost for the neutralizing antibody responses and cellular responses that cross-recognize Delta and Omicron variants, compared to the two doses of vaccine. Moreover, the potency, breadth, and duration of adaptive responses improved concomitantly. Nevertheless, the data also underlined the need for continued surveillance and the potential danger posed by continued variants evolution that resulted in further reduction of adaptive immune responses. The incorporation of additional elements eliciting broader adaptive immune responses directed towards more conserved targets into vaccine strategies may be considered a means to increase vaccine effectiveness against future variants.

Conflict of Interest Statement

The authors have declared that no conflict of interest exists.

Author contributions statement

CW and HS designed the study. YC, LC, YT recruited the patients. ML, YW and JN processed the blood samples, MM, LM and YS performed cellular analysis. YL performed the antibody assay. YS and CL analyzed and interpreted the data. YC and LC wrote the manuscript. All the authors revised the manuscript.

Acknowledgements

We thank all vaccine recipients for providing serum samples. This study was supported by Clinical Trials from the Affiliated Drum Tower Hospital, Medical School of Nanjing University (2021-LCYJ-PY-09), Nanjing Important Science & Technology Specific Projects (2021-11005), Nanjing Medical Science and Technique Development Foundation (QRX17141 and YKK19056), Social Development Foundation of Jiangsu Province（BE2021739).

References

1. Amit S, Gonen T, Regev-Yochay G. Covid-19 Breakthrough Infections in Vaccinated Health Care Workers. Reply. N Engl J Med. 2021 Oct 21;385(17):1630-1631.

2. Goldberg Y, Mandel M, Bar-On YM, et al. Waning Immunity after the BNT162b2 Vaccine in Israel. N Engl J Med. 2021 Dec 9;385(24):e85.

3. Espenhain L, Funk T, Overvad M, et al. Epidemiological characterisation of the first 785 SARS-CoV-2 Omicron variant cases in Denmark, December 2021. Euro Surveill. 2021 Dec;26(50).

4. Centers for Disease Control and Prevention. CDC COVID Data Tracker. (2022). Available online at: https://covid.cdc.gov/covid-data-tracker/#variant-proportions

5. Wang Y, Zhang L, Li Q, et al. The significant immune escape of pseudotyped SARS-CoV-2 variant Omicron. Emerg Microbes Infect. 2022 Dec;11(1):1-5.

6. Krause PR, Fleming TR, Peto R, et al. Considerations in boosting COVID-19 vaccine immune responses. Lancet. 2021 Oct 9;398(10308):1377-1380.

7. Callaway E. COVID vaccine boosters: the most important questions. Nature. 2021 Aug;596(7871):178-180.

8. Kalafat E, Magee LA, von Dadelszen P, et al. COVID-19 booster doses in pregnancy and global vaccine equity. Lancet. 2022 Mar 5;399(10328):907-908.

9.SAGE. Highlights from the meeting of the Strategic Advisory Group of Experts (SAGE) on Immunization. Oct 11, 2021.

10.Zeng G, Wu Q, Pan H, et al. Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials. Lancet Infect Dis. 2022 Apr;22(4):483-495.

11.Souza WM, Amorim MR, Sesti-Costa R, et al. Neutralisation of SARS-CoV-2 lineage P.1 by antibodies elicited through natural SARS-CoV-2 infection or vaccination with an inactivated SARS-CoV-2 vaccine: an immunological study. Lancet Microbe. 2021 Oct;2(10):e527-e535.

12.Ranzani OT, Hitchings MDT, Dorion M, et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study. BMJ. 2021 Aug 20;374:n2015.

13.Bar-On YM, Goldberg Y, Mandel M, et al. Protection against Covid-19 by BNT162b2 Booster across Age Groups. N Engl J Med. 2021 Dec 23;385(26):2421-2430.

14.Bar-On YM, Goldberg Y, Mandel M, et al. Protection of BNT162b2 Vaccine Booster against Covid-19 in Israel. N Engl J Med. 2021 Oct 7;385(15):1393-1400.

15.Ramasamy MN, Minassian AM, Ewer KJ, et al. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. Lancet. 2021 Dec 19;396(10267):1979-1993.

16.Hotez PJ, Bottazzi ME. Whole Inactivated Virus and Protein-Based COVID-19 Vaccines. Annu Rev Med. 2022 Jan 27;73:55-64.

17.Stamatatos L, Czartoski J, Wan YH, et al. mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. Science. 2021 Mar 25;eabg9175. doi: 10.1126/science.abg9175.

18.Garcia-Beltran WF, St Denis KJ, Hoelzemer A, et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. Cell. 2022 Feb 3;185(3):457-466 e4.

19.Pajon R, Doria-Rose NA, Shen X, et al. SARS-CoV-2 Omicron Variant Neutralization after mRNA-1273 Booster Vaccination. N Engl J Med. 2022 Mar 17;386(11):1088-1091.

20.Chen Y, Shen H, Huang R, et al. Serum neutralising activity against SARS-CoV-2 variants elicited by CoronaVac. Lancet Infect Dis. 2021 Aug;21(8):1071-1072.

21.Chen Y, Yin S, Tong X, et al. Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine. Clin Microbiol Infect. 2022 Mar;28(3):410-418.

22. Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. Nat Biotechnol. 2020 Sep;38(9):1073-1078.

23.Li T, Han X, Gu C, et al. Potent SARS-CoV-2 neutralizing antibodies with protective efficacy against newly emerged mutational variants. Nat Commun. 2021 Nov 2;12(1):6304.

24.Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020 Mar 13;367(6483):1260-1263.

25. Dan JM, Lindestam Arlehamn CS, et al. A Cytokine-Independent Approach To Identify Antigen-Specific Human Germinal Center T Follicular Helper Cells and Rare Antigen-Specific CD4+ T Cells in Blood. J Immunol. 2016 Aug 1;197:983-93.

26. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science. 2021 Jan 6: eabf4063.

27. Painter MM, Mathew D, Goel RR, et al. Rapid induction of antigen-specific CD4(+) T cells is associated with coordinated humoral and cellular immunity to SARS-CoV-2 mRNA vaccination. Immunity. 2021 Sep14; 54:2133-2142 e3.

28.Bates TA, Leier HC, Lyski ZL, et al. Age-Dependent Neutralization of SARS-CoV-2 and P.1 Variant by Vaccine Immune Serum Samples. JAMA. 2021 Jul 21;326(9):868-869.doi: 10.1001/jama.2021.11656.

29.Qi H. T follicular helper cells in space-time. Nat Rev Immunol. 2016 Oct;16(10):612-25.

30.Crotty S. Follicular helper CD4 T cells (TFH). Annu Rev Immunol. 2011;29:621-63.

31.Morita R, Schmitt N, Bentebibel SE, et al. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. Immunity. 2011 Jan 28;34(1):108-21.

32.Schmitt N, Bentebibel SE, Ueno H. Phenotype and functions of memory Tfh cells in human blood. Trends Immunol. 2014 Sep;35(9):436-42.

33.Tarke A, Sidney J, Methot N, et al. Impact of SARS-CoV-2 variants on the total CD4(+) and CD8(+) T cell reactivity in infected or vaccinated individuals. Cell Rep Med. 2021 Jul 20;2(7):100355.

34.Geers D, Shamier MC, Bogers S, et al. SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. Sci Immunol. 2021 May 25;6(59):eabj1750.

doi: 10.1126/sciimmunol.abj1750.

35.Liu L, Iketani S, Guo Y, et al. Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. Nature. 2022 Feb;602(7898):676-681.

36.Dejnirattisai W, Shaw RH, Supasa P, et al. Reduced neutralisation of SARS-CoV-2 omicron B.1.1.529 variant by post-immunisation serum. Lancet. 2022 Jan 15;399(10321):234-236.

37.Sterlin D, Mathian A, Miyara M, et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. Sci Transl Med. 2021 Jan 20;13(577).

38.Bates TA, McBride SK, Winders B, et al. Antibody Response and Variant Cross-Neutralization After SARS-CoV-2 Breakthrough Infection. JAMA. 2022 Jan 11;327(2):179-181.

39.Goel RR, Painter MM, Apostolidis SA, et al. mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern. Science. 2021 Dec 3;374(6572):abm0829.

40.Brenna E, Davydov AN, Ladell K, et al. CD4(+) T Follicular Helper Cells in Human Tonsils and Blood Are Clonally Convergent but Divergent from Non-Tfh CD4(+) Cells. Cell Rep. 2020 Jan 7;30(1):137-152 e5.

41.Juno JA, Tan HX, Lee WS, et al. Humoral and circulating follicular helper T cell responses in recovered patients with COVID-19. Nat Med. 2020 Sep;26(9):1428-1434.

42.Mudd PA, Minervina AA, Pogorelyy MV, et al. SARS-CoV-2 mRNA vaccination elicits a robust and persistent T follicular helper cell response in humans. Cell. 2022 Feb 17;185(4):603-613 e15.

43.Farooq F, Beck K, Paolino KM, et al. Circulating follicular T helper cells and cytokine profile in humans following vaccination with the rVSV-ZEBOV Ebola vaccine. Sci Rep. 2016 Jun 21;6:27944.

44.Tarke A, Sidney J, Methot N, et al. Impact of SARS-CoV-2 variants on the total CD4(+) and CD8(+) T cell reactivity in infected or vaccinated individuals. Cell Rep Med. 2021 Jul 20;2(7):100355.

45.Liu J, Chandrashekar A, Sellers D, et al. Vaccines elicit highly conserved cellular immunity to SARS-CoV-2 Omicron. Nature. 2022 Mar;603(7901):493-496.

46.Keeton R, Tincho MB, Ngomti A, et al. T cell responses to SARS-CoV-2 spike cross-recognize Omicron. Nature. 2022 Mar;603(7901):488-492.

47.Tarke A, Coelho CH, Zhang Z, et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. Cell. 2022 Mar 3;185(5):847-859 e11.

48.Wolter N, Jassat W, Walaza S, et al. Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study. Lancet. 2022 Jan 29;399(10323):437-446.

Figure legends

Figure 1. Study design and cohort summary.

Figure 2. Dynamic anti-RBD or anti-spike antibody responses before and after the third CoronaVac booster. (A-D) Enzyme-linked immunosorbent assay measurement for anti-RBD IgG titer **(A),** anti-RBD IgA titer **(B),** anti-spike IgG titer **(C)** and anti-spike IgA titer **(D)** at three different time points, including before the booster (T0), 2 weeks after the booster (T1), and 8 weeks after the booster (T2) for vaccine booster group. Serum from a breakthrough cohort was also included for analysis as control, which were obtained between day 13-18 post disease onset. Dotted lines indicated the lower limit of detection (LOD) for the assay. Data points on the bar graph represent individual titer and the line indicates geometric mean titer (GMT). GMTs and the seropositive ratios were noted on the top of each bar. **(E-H)** Fold change in anti-RBD IgG titer **(E),** anti-RBD IgA titer**(F),** anti-spike IgG titer **(G)**, anti-spike IgA titer **(H)** specific to Delta or Omicron compared to that of ancestral strain for booster at T1 and T2 timepoint as well as for breakthrough cohort. For comparing antibody responses specific to different SARS-CoV-2 variants and at timepoints, two-tailed p values were determined using matched-pairs signed rank test with the Holm-Šídák multiple comparison correction. Unpaired wilcoxon test for comparison between vaccine booster at T1 timepoint and breakthrough infection subjects. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns, no significant difference.

Figure 3. Serum neutralization activities in CoronaVac booster recipients and breakthrough infection cases. (A) Serum titers that achieved 50% peudovirus neutralization (ID_{50}) in 77 CoronaVac booster recipients and 10 Delta breakthrough infection cases with prior 2-dose of CoronaVac. The vaccine sera were collected on day 14 post 2-dose and 3-dose of CoronaVac,

respectively, while the breakthrough sera were obtained between day 13-18 post disease onset. The horizontal dotted lines indicate half the value of the lower limit of detection. Data points showed on the bar graph represent individual titer and the line indicates geometric mean titer (GMT). For pairwise comparison of serum samples collected after 2 doses and 3 doses, two-tailed *p* values were determined using matched-pairs signed rank test with the Holm-Šídák multiple comparison correction. Unpaired Wilcoxon test was used for comparison between groups. **(B)** Fold change in neutralization titer (ID_{50}) for Delta and Omicron strain relative to that for D614G strain at different timepoint. The mean change fold was on the top of bar. **(C)** Correlation analysis of anti-RBD IgA or IgG responses specific to ancestral, Delta, and Omicron. **(D)** Correlation analysis of neutralization titer (ID_{50}) against D614, Delta, and Omicron strain, correlation analysis of ID_{50} against Delta strain and anti-Delta spike IgG, and correlation analysis of ID_{50} against Omicron strain and anti-Omicron spike IgG or anti-Delta spike IgG responses. **(E)** Neutralization titers against D614G, Delta and Omicron strain among booster recipients under the age of 40 versus over the age of 40. **(F)** Neutralization titers against D614G, Delta and Omicron strains among low neutralizers after 2-dose CoronaVac versus high neutralizers after 2-dose CoronaVac. Correlation analysis for neutralization titers after 2 doses versus neutralization titers after 3 doses for D614G, Delta and Omicron strain. Correlation analysis was performed using nonparametric Spearman rank correlation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns, no significant difference.

Figure 4. RBD-specific B cell responses and memory B cell subsets in vaccine cohort. (A) The frequency of RBD-specific B cells of total B cells over time in booster recipients at T0, T1 and T2 timepoint, and the frequency of B cells specific to ancestral RBD, Delta RBD, and Omicron RBD at T2 timepoint. Dotted lines indicated the limit of detection (LOD) for the assay. **(B)** The frequency of RBD-specific memory B cells of total memory B cells over time in booster recipients

at T0, T1 and T2 timepoint. The frequency of memory B cells specific to ancestral RBD, Delta RBD, and Omicron RBD at T2 timepoint. **(C-D)** Fold change for the frequency of RBD-specific B cells **(C)** and RBD-specific memory B cells **(D)** recognizing Delta and Omicron strain relative to that of counterpart recognizing ancestral strain. Bars represent the average frequency of B cells, and positive rate was on the top of each bar. Wilcoxon matched-pairs signed rank with two-tailed p value was used for comparison between groups. * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001; ns, no significant difference.

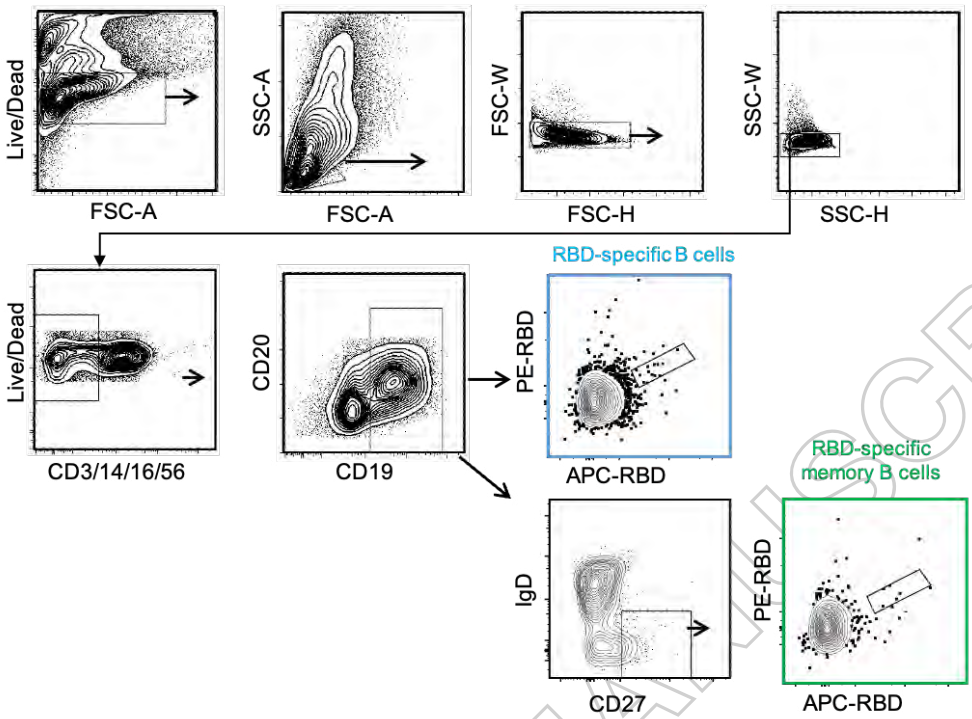
Figure 5. Spike-specific circulating follicular helper cell (cTfh) responses in vaccine cohort. **(A)** The frequency of cTfh responding to ancestral spike, Alpha spike, Delta spike and Omicron spike (T2 only) of total cTfh cells over time in booster recipients. Dotted lines indicate the lower limit of detection (LOD) for the assay. **(B)** Fold change for the frequency of cTfh cells recognizing Alpha, Delta and Omicron strain relative to that of counterpart recognizing ancestral strain. **(C)** Correlation analyses of Omicron spike-specific cTfh cells and ancestral spike-specific cTfh cells, Alpha Spike-specific cTfh cells, and Omicron spike-specific cTfh cells, respectively. **(D)** Dynamic change of spike-specific cTfh subpopulations at T0, T1 and T2 timepoint, including cTfh1, cTfh2, cTfh17 and cTfh1-17. Bars represent the average frequency of cTfh cells, and positive rate was on the top of each bar. Two-tailed p values were determined using matched-pairs signed rank test with the Holm-Šídák multiple comparison correction between groups. * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001; ns, no significant difference.

Figure 6. SARS-CoV-2 specific $CD4^+$ and $CD8^+$ T cell responses in vaccine cohort. (A-B) The frequency of SARS-CoV-2 specific $CD4^+$ T cell **(A)** and $CD8^+$**(B)** T cell responses over time in booster recipients. The frequency of $CD4^+$**(A)** and $CD8^+$**(B)** T cell responses responding to

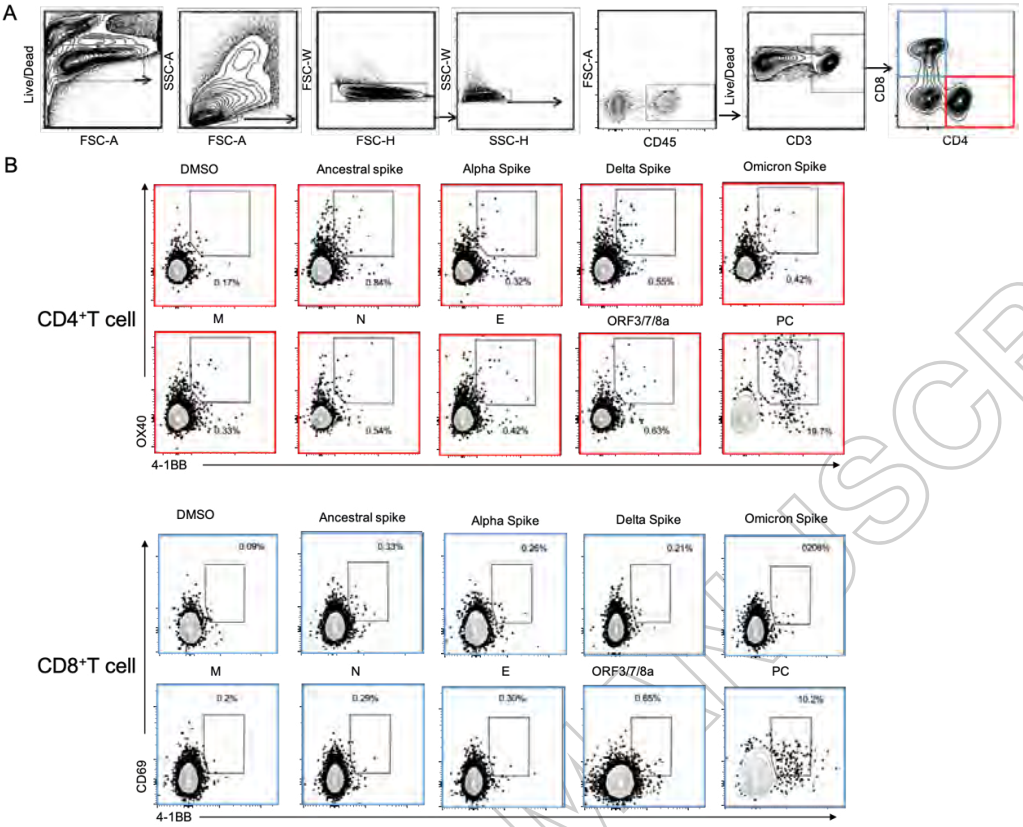
ancestral spike (T0-T2), Alpha spike (T0-T2), Delta spike(T0-T2), and Omicron spike (T2 only) over time in booster recipients. Dotted lines indicated the limit of detection (LOD) for the assay. **(C-D)** Fold change for the frequency of $CD4^+$ T cells **(C)** or $CD8^+$ T cells **(D)** recognizing Alpha, Delta and Omicron strain relative to that of counterpart recognizing ancestral strain. **(E)** Comparative analysis for the frequency of $CD4^+$ T cells or $CD8^+$ T cells specific to Delta strain among those booster recipients who do not generate neutralization antibody responses against Delta strain (Delta NAb non-responders) versus Delta NAb responders (left panel). Comparative analysis for the frequency of $CD4^+$T cells or $CD8^+$ T cells specific to Omicron strain among those booster recipients who do not generate neutralization activities against Omicron strain (Omicron NAb non-responders) versus Omicron NAb responders (right panel). Bars represented median value, whereas median value and positive rate was on the top of each bar. When comparing T cell responses specific to different SARS-CoV-2 variants and at timepoints, two-tailed p values were determined using matched-pairs signed rank test with the Holm-Šídák multiple comparison correction. Unpaired wilcoxon test were used for comparison between vaccine booster at T1 timepoint and breakthrough infection subjects. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns, no significant difference.

Table 1. Baseline clinical characteristics of the CoronaVac booster cohort and the breakthrough infection cohort.

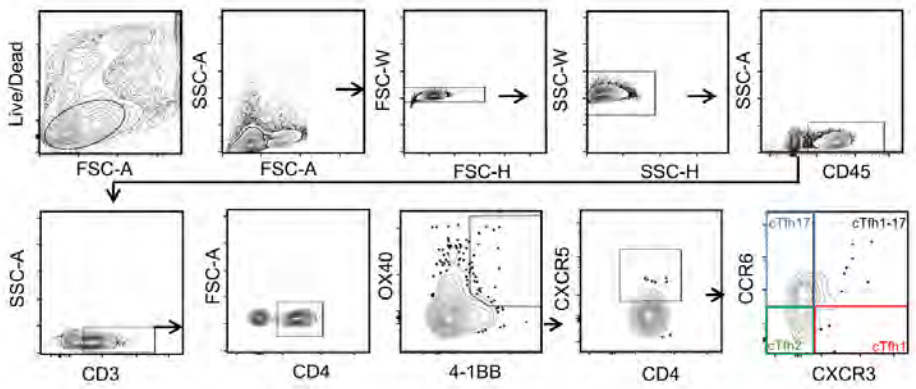
	CoronaVac booster group (n=77)	**breakthrough infection group** (n=10)
Sex		
Male	32(41.6)	2(20%)
Female	45(58.4)	8(80%)
Age (years)		
Median age (IQR)	35.0(28.3, 40.0)	45.0(44.3, 47.8)
Age group, years		
18-29	26(33.8)	0(0%)
30-39	30(39.0)	0(0%)
40-49	14(18.1)	9(90%)
50-59	7(9.1)	1(10%)
Sample types	serum and PBMC	serum
Interval between 1st and 2nd dose of CoronaVac(days)		
Median (IQR)	21(17.25,22.75)	22(17.0,38.5)
Booster or infection since the 2nd dose of CoronaVac (months)		
Median (IQR)	8.43(8.03,8.52)	2.37(1.33,3.73)



Supplemental Figure 1. Gating strategies to define SARS-CoV-2 RBD-specific B cells. Live cells were identified as Live/Dead$^-$ and lymphocytes were then gated based on forward- and side-scatter. Doublets were then excluded by FSC-W vs. FSC-H and SSC-W vs. SSC-H. Total B cells were identified as CD3$^-$CD14$^-$CD16$^-$CD56$^-$CD19$^+$CD20$^+$cells. Memory B cells were identified as CD20$^+$CD27$^+$cells. RBD specific B cells were identified based on binding to corresponding RBD probes.



Supplemental Figure 2. Gating strategies to define SARS-CoV-2 specific T cells. (A) Live cells were identified as Live/Dead$^-$ and lymphocytes were gated based on forward- and side-scatter. Doublets were then excluded by FSC-W vs. FSC-H and SSC-W vs. SSC-H. Total T cells were identified as CD45$^+$CD3$^+$, which were further divided into CD8$^+$ and CD4$^+$ subsets. **(B)** Representative examples of flow cytometry plots of SARS-CoV-2-specific CD4$^+$ and CD8$^+$ T cells by activation-induced marker (AIM) assay. AIM$^+$CD4$^+$ T cells were identified based on dual expression of 4-1BB and OX40, while AIM$^+$CD8$^+$ T cells were identified based on dual expression of 4-1BB and CD69, after overnight stimulation with indicated peptide pools including ancestral spike, Alpha Spike, Delta Spike, Omicron Spike, Membrane (M), Nucleocapsid (N), membrane protein (M), ORF3/7/8a peptide pools or PMA/Ionomycin as positive control (PC), compared to negative control stimulation (DMSO).



Supplemental Figure 3. Gating strategies to define SARS-CoV-2 spike specific circulating Tfh (cTfh) cells. Live cells were identified as Live/Dead$^-$ and lymphocytes were gated based on forward- and side-scatter. Doublets were then excluded by FSC-W vs. FSC-H and SSC-W vs. SSC-H. Total T cells were identified as CD45$^+$CD3$^+$. After overnight stimulation with the indicated spike peptide pools, CD4$^+$ T cells with the dual expression of 4-1BB and OX40 were considered as AIM$^+$CD4$^+$ T cells. Spike-specific cTfh cells were gated as CXCR5$^+$4-1BB$^+$OX40$^+$CD4$^+$ cells and further divided into Tfh1(CXCR3$^+$CCR6$^-$), Tfh2(CXCR3$^-$CCR6$^-$), Tfh17(CXCR3$^-$CCR6$^+$) and Tfh1-17(CXCR3$^+$CCR6$^+$).

Figure 1

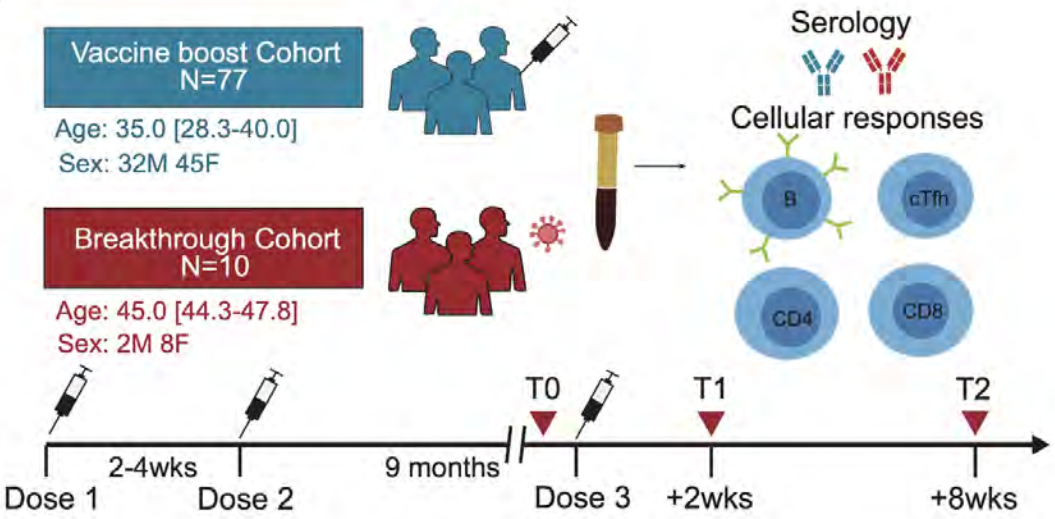


Figure 2

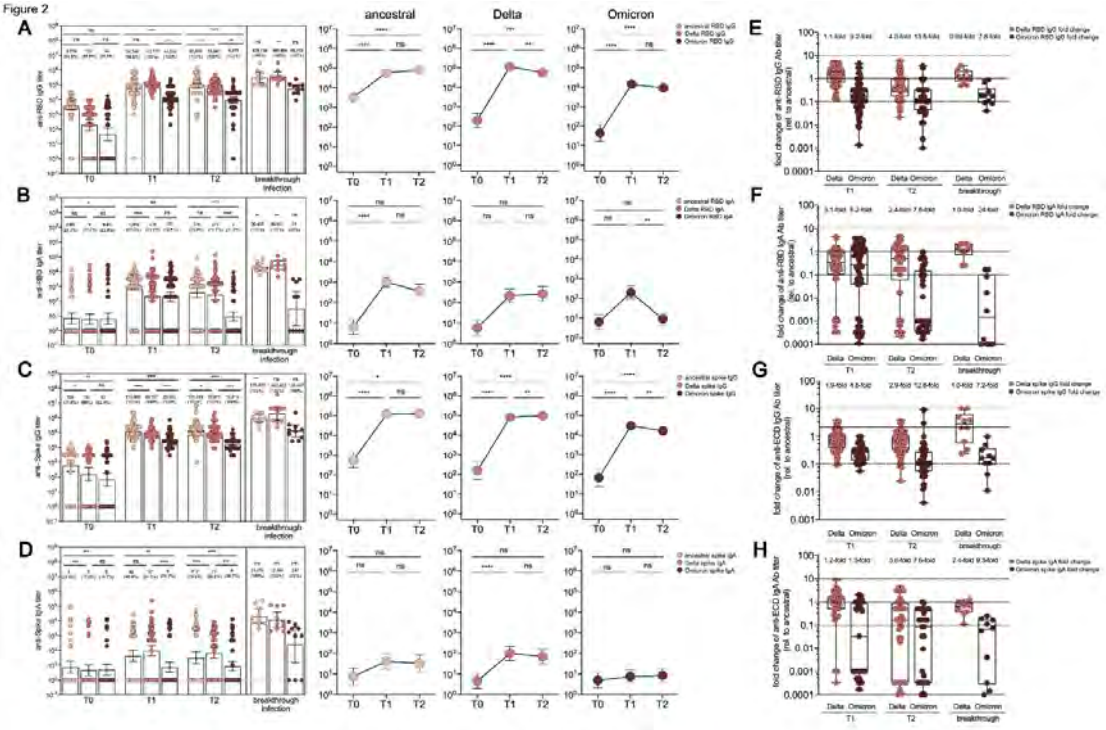


Figure 3

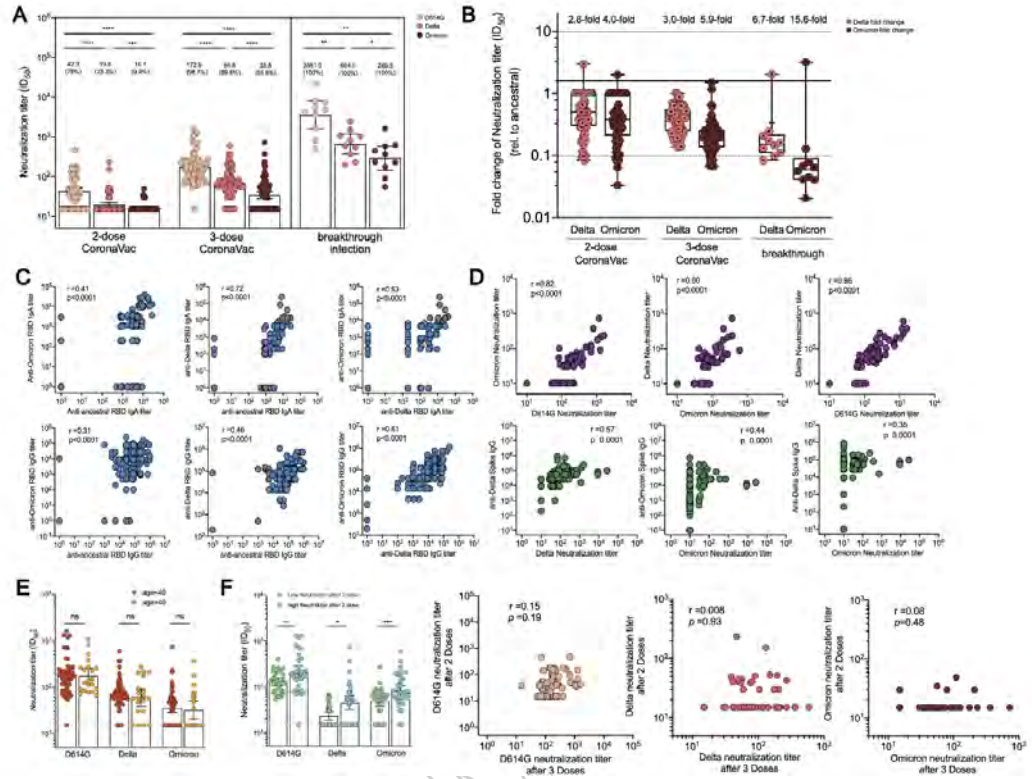


Figure 4

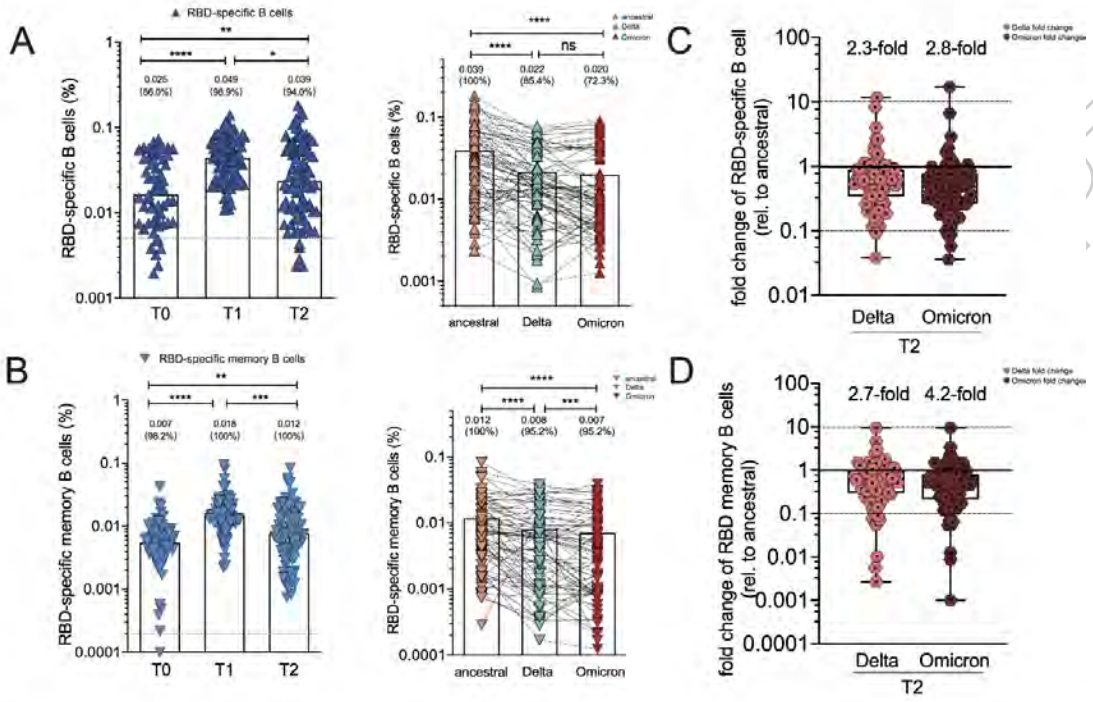


Figure 5

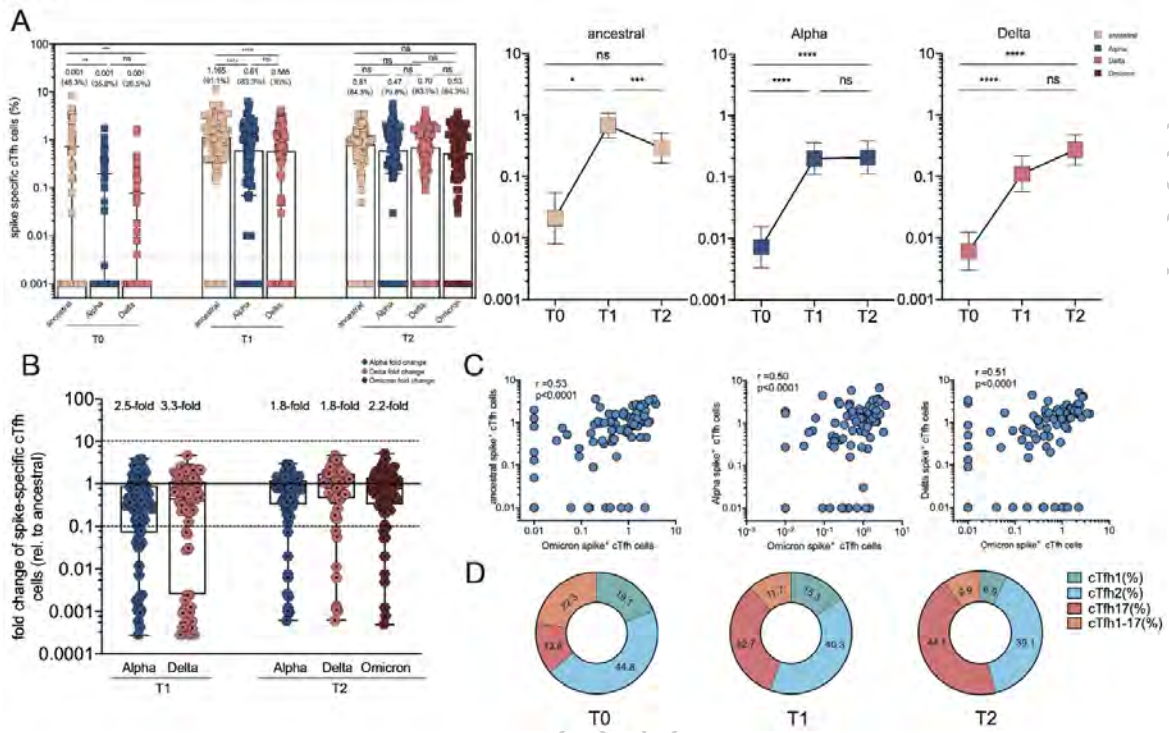
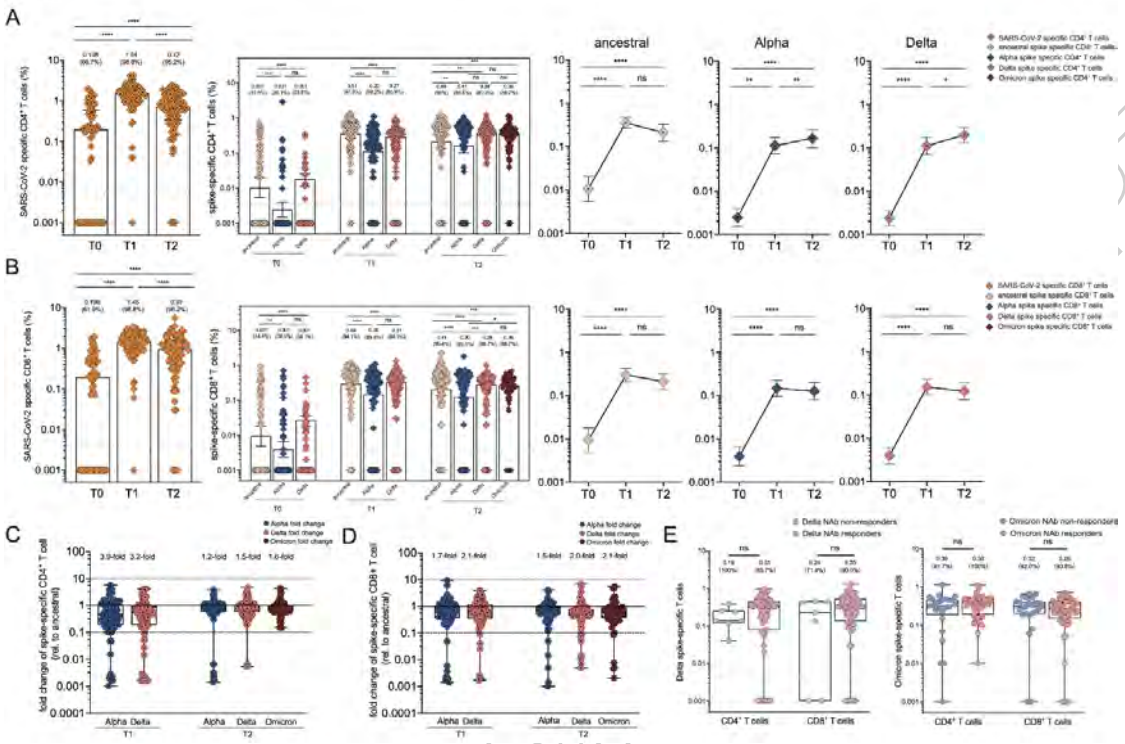


Figure 6



7.5. Estudo com 11 milhões de chilenos mostra eficácia da dose de reforço da CoronaVac acima de 85% contra casos graves

Um estudo feito por pesquisadores chilenos e publicado na plataforma de preprints SSRN, vinculada à revista científica The Lancet, mostrou que a administração da dose de reforço da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, apresentou uma eficácia de 78,8% para casos sintomáticos, 86,3% para hospitalizações, 92,2% para internações em Unidades de Terapia Intensiva (UTIs) e 86,7% para evitar mortes relacionadas à Covid-19.

Esse é o maior estudo já feito sobre a eficácia da dose de reforço da CoronaVac e foi conduzido por pesquisadores do Ministério da Saúde do Chile, da Faculdade de Medicina da Pontifícia Universidade Católica do Chile, do Instituto Millenium e das faculdades de Medicina e de Saúde Pública da Universidade Harvard.

A pesquisa foi realizada entre fevereiro e novembro de 2021 e envolveu 11,2 milhões de pessoas (58% da população do Chile), com idades a partir dos 16 anos, que haviam completado o esquema vacinal primário com CoronaVac e tomado a dose de reforço do mesmo imunizante. Na época, a variante delta era predominante no Chile.

Segundo os cientistas, os resultados "sugerem que uma dose de reforço da CoronaVac para indivíduos com esquema completo de vacinação primária com CoronaVac fornece um alto nível de proteção contra a Covid-19, incluindo doença grave e morte".

O estudo, publicado sob o nome "Effectiveness of Homologous and Heterologous Booster Shots for an Inactivated SARS-CoV-2 Vaccine: A Large-Scale Observational Study", avaliou a eficácia das defesas imunológicas induzidas nas pessoas que tomaram três doses da CoronaVac na comparação com pessoas ainda não vacinadas. Foram excluídos participantes com histórico prévio de infecção por Covid-19.

Publicado em: 13/01/2022

Effectiveness of homologous and heterologous booster shots for an inactivated SARS-CoV-2 vaccine: A large-scale observational study

Alejandro Jara Ph.D.[1,2,3], Eduardo A. Undurraga Ph.D.[4,5,6,7], José R. Zubizarreta Ph.D.[8,9,10], Cecilia González M.D.[1], Alejandra Pizarro M.D.[1], Johanna Acevedo M.S.[1], Katherine Leo B.S.E.[1], Fabio Paredes M.Sc.[1], Tomás Bralic M.S.[1], Verónica Vergara, M.S.[1], Marcelo Mosso B.S.E.[1], Francisco Leon M.B.A.[1], Ignacio Parot, M.B.A.[1], Paulina Leighton B.S.E.[1], Pamela Suárez B.S.E[1], Juan Carlos Rios Ph.D.[1,11], Heriberto García-Escorza M.S.[1], and Rafael Araos, M.D.[1,5,12,13]*

[1] *Ministry of Health, Enrique Mac Iver 541, Santiago, Región Metropolitana, Chile*

[2] *Facultad de Matemáticas, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Macul, Santiago, Región Metropolitana, Chile*

[3] *Millennium Nucleus Center for the Discovery of Structures in Complex Data (MiDaS), Av. Vicuña Mackenna 4860, Macul, Santiago, Región Metropolitana, Chile*

[4] *Escuela de Gobierno, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Macul, Santiago, Región Metropolitana, Chile*

[5] *Millennium Initiative for Collaborative Research in Bacterial Resistance (MICROB-R), Av. las Condes 12496, Lo Barnechea, Santiago, Región Metropolitana, Chile*

[6] *Research Center for Integrated Disaster Risk Management (CIGIDEN), Av. Vicuña Mackenna 4860, Macul, Santiago, Región Metropolitana, Chile*

[7] *CIFAR Azrieli Global Scholars program, CIFAR, 661 University Ave., Toronto, ON M5G 1M1 Canada*

[8] *Department of Health Care Policy, Harvard Medical School, 25 Shattuck St, Boston, MA 02115, United States*

[9] *Department of Biostatistics, Harvard T.H. School of Public Health, 25 Shattuck St, Boston, MA 02115, United States*

[10] *Department of Statistics, Harvard T.H. School of Public Health, 25 Shattuck St, Boston, MA 02115, United States*

[11] *Facultad de Medicina, Pontificia Universidad Católica de Chile, Lira 40, Santiago, Región Metropolitana*

[12] *Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina, Universidad del Desarrollo, Av. las Condes 12496, Lo Barnechea, Santiago, Región Metropolitana, Chile*

[13] *Advanced Center for Chronic Diseases (ACCDiS), Sergio Livingstone 1007, Independencia, Santiago, Región Metropolitana, Chile*

***Correspondence to** Dr. Rafael Araos at Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina Clínica Alemana Universidad del Desarrollo, Av. Las Condes 12461, Las Condes, Región Metropolitana, Chile. rafaelaraos@udd.cl

Abstract

Background. Vaccine protection against Covid-19 may be waning. Several countries have authorized or begun using a booster vaccine dose. Policymakers urgently need evidence of the effectiveness of additional vaccine doses against Covid-19 and its clinical spectrum for individuals with complete primary immunization schedules.

Methods. We used a prospective national cohort of 11·2 million persons 16 years or older to assess the effectiveness of CoronaVac, AZD1222, or BNT162b2 vaccine boosters in individuals who completed their primary immunization schedule with CoronaVac compared to unvaccinated individuals. The study was conducted in Chile from February 2 through November 10, 2021. We used inverse probability-weighted survival regression models to estimate hazard ratios, accounting for time-varying vaccination status and adjusting for relevant demographic, socioeconomic, and clinical confounders. We estimated the change in the hazard associated with complete immunization (≥14 days after the booster).

Findings. We found an adjusted vaccine effectiveness against symptomatic Covid-19 of 78·8% (95% confidence interval, CI, 76·8–80·6) for a three-dose schedule with CoronaVac, 96·5% (95% CI, 96·2–96·7) for BNT162b2 booster, and 93·2% (95% CI, 92·9–93·6) for the AZD1222 booster. The adjusted vaccine effectiveness against hospitalization, ICU admission, and Covid-19 related deaths was 86·3%, 92·2%, and 86·7% for a three-dose schedule with CoronaVac, 96·1%, 96·2%, and 96·8% for the BNT162b2 booster, and 97·7%, 98·9%, and 98·1% for the AZD1222 booster.

Interpretation. Our results suggest that a homologous or heterologous booster shot for individuals with a complete primary vaccination schedule with CoronaVac provides a high level of protection against Covid-19, including severe disease and death. However, heterologous boosters showed higher vaccine effectiveness for all outcomes, providing additional support for using a mix and match approach.

Funding. Agencia Nacional de Investigación y Desarrollo (ANID) Millennium Science Initiative Program and Fondo de Financiamiento de Centros de Investigación en Áreas Prioritarias (FONDAP).

Keywords: SARS-CoV-2, Covid-19, vaccine effectiveness, inactivated SARS-CoV-2 vaccine, immunization program, boosters.

Word count

Text: 3498/3500, Abstract: 298/300, Tables and Figures: 3, References 31/30

Research in context

Evidence before this study

We searched PubMed and medRxiv for research articles, with no language restrictions, using the search terms ("Covid-19" OR "SARS-CoV-2" OR "2019-nCoV" OR "coronavirus") AND ("vaccine" OR "vaccination") AND ("third dose" OR "booster"). We searched for studies published between December 1, 2020, and December 10, 2021. We identified five original clinical studies on the effectiveness of booster shots for SARS-CoV-2 vaccines. Four studies from Israel have examined the effectiveness of a third dose Pfizer-BioNTech's mRNA vaccine BNT162b2 compared to the primary vaccination series. One study estimated a 70-84% reduction in the probability of testing positive for SARS-CoV-2 infection among individuals with a third dose but did not examine other clinical outcomes. Another study found that the rate of infection in the booster group was lower by a factor of 11.3 for confirmed infection and 19.5 for severe illness but did not adjust for comorbidities. The third study found a 90% lower Covid-19 related mortality among participants with a third vaccine dose. The fourth study estimated that the vaccine effectiveness of the third dose of BNT162b2 was 93%, 92%, and 81% against admission to the hospital, severe disease, and death. Last, a preprint study found that, compared with demographically and clinically matched individuals with two doses, the effectiveness of a third dose in preventing SARS-CoV-2 infection and hospitalization was 46% and 47% for BNT162b2 and 47% and 50% for Moderna's mRNA-1273. All available evidence relates to mRNA vaccines. We found no studies examining the effectiveness of a homologous or heterologous booster shot for individuals with a complete primary vaccination schedule with an inactivated SARS-CoV-2 vaccine. These estimates are essential for policymakers.

Added value of this study

Our study estimates the effectiveness of a homologous or heterologous booster shot for individuals with a complete primary vaccination schedule with an inactivated SARS-CoV-2 vaccine, which accounts for about half the Covid-19 vaccine doses delivered globally. Specifically, we used a prospective national cohort of 11·2 million persons 16 years or older to assess the effectiveness of CoronaVac, AZD1222, or BNT162b2 vaccine boosters in individuals who completed their primary immunization schedule with CoronaVac against symptomatic Covid-19, hospitalization, admission to ICU, and death, adjusting for known demographic, socioeconomic, and clinical confounders by inverse probability of treatment weighting.

Implications of all the available evidence

Covid-19 vaccines are an essential component of the pandemic response to reduce disease burden. However, growing evidence suggests that vaccine protection against Covid-19 may be waning over time or have lower effectiveness against the Delta variant (B-1-617.2). The decrease in vaccine protection is particularly worrying for inactivated vaccines, which offer lower protection than other vaccine technologies. In light of this emerging evidence, several countries have authorized or begun using a third dose. Our results suggest that a homologous or heterologous booster shot for individuals with a complete primary vaccination schedule with CoronaVac provides a high level of protection against Covid-19, including severe disease and death.

Background

The coronavirus disease 2019 (Covid-19) pandemic has had a major global impact, with more than 250 million cases and 5 million deaths reported globally as of November 10, 2021.[1] Covid-19 vaccines are now an essential component of the pandemic response to reduce disease burden and allow a safer reopening of society and economic recovery. Twenty-three effective coronavirus vaccines have been approved for use since the first vaccine tested in a large randomized clinical trial was approved in the United Kingdom on December 2, 2020,[2] and several new vaccines are in the final testing stage.[3] The efficacy, effectiveness, and safety profiles of numerous vaccine platforms are well-supported by large-scale efficacy trials or observational studies.[3] Many countries are currently running mass vaccination campaigns.[4]

Growing evidence suggests that vaccine protection against Covid-19 may be waning over time, and newly emerging variants, such as Delta and Omicron, may evade vaccine-induced immune protection for some vaccines.[5,6] While the correlates of protection for Covid-19 vaccines are not fully understood,[7,8] research has shown a time-dependent decline in humoral immune responses,[9,10] which may parallel decreasing protection against infection and disease. Research suggests that this decline also occurs for Sinovac's CoronaVac inactivated SARS-CoV-2 vaccine.[11,12] There have also been increased reports of breakthrough infections among vaccinated individuals.[13-15] Recent research has shown that vaccine effectiveness may fade over time, particularly for symptomatic illness.[6,16,17] These studies have examined data for Pfizer-BioNTech's mRNA vaccine BNT162b2 and Oxford-AstraZeneca's ChAdOx1 nCov-19 AZD1222 vaccine recipients, and it is still unclear whether protection against more severe disease has decreased as well. A potential decrease in vaccine protection is particularly worrying for inactivated vaccines, which offer lower protection than other vaccine technologies, and account for about half the Covid-19 vaccine doses delivered globally thus far.[18]

In light of this emerging evidence, several high-income countries, including France, Germany, Israel, the United States, and the United Kingdom, have authorized or begun using a third vaccine dose.[19] Most have limited vaccine boosters to persons at higher risk, including older adults, healthcare workers, and individuals with underlying health conditions. Other countries that have relied on inactivated vaccines, including Cambodia, Chile, Uruguay, Thailand, and Turkey offer homologous or heterologous booster vaccine shots to individuals immunized with inactivated

vaccines SARS-CoV-2 vaccine schedules.[19] Policymakers urgently need evidence of the effectiveness of vaccine boosters against severe disease for individuals that have completed their primary immunization schedules. Existing evidence for the effectiveness of boosters is limited to mRNA vaccines.[20-24]

On February 2, 2021, Chile began a mass vaccination campaign based on four Covid-19 vaccines. The Ministry of Health organized vaccination rollout through a publicly available schedule at the national level, assigning specific dates to eligible groups.[25] On August 11, 2021, the Ministry of Health began administering a booster dose for individuals fully vaccinated with the CoronaVac Covid-19 vaccine. CoronaVac has been the campaign's backbone, with 59·0% (20·5 million) of all doses administered as of November 16, 2021. Pfizer-BioNTech's BNT162b2 represents 30·5% (10·6 million) of doses, and Oxford-AstraZeneca's AZD1222 vaccine and CanSino Biologics' Ad5-nCoV vaccine represent 8·8% (3·1M) and 1·7% of doses (0·57), respectively.[25]

We use a rich administrative dataset of 11·2 million individuals to assess the effectiveness of CoronaVac, AZD1222, or BNT162b2 vaccine boosters (third doses) in preventing Covid-19 cases, hospitalizations, admission to the intensive care unit (ICU), and deaths in individuals who completed their primary immunization schedule with CoronaVac. We estimate vaccine effectiveness for homologous (three-dose schedule) and heterologous (mix and match) booster shots adjusting for relevant demographic and clinical confounders of the association between vaccination and Covid-19 outcomes. Our results are relevant to policymakers considering a third dose for populations vaccinated with CoronaVac, the most widely used Covid-19 vaccine globally,[18] and provide essential information on homologous and heterologous vaccine booster schedules.

Methods

Study population and design

We used data from a prospective observational national-level cohort including 11·2 million participants aged 16 or older affiliated with the Fondo Nacional de Salud (FONASA). FONASA is the national health insurance program that collects, manages, and distributes funds for the healthcare system. Eligibility criteria included being 16 years of age or older, affiliated with FONASA (about 80% of the Chilean population), and vaccinated with CoronaVac, BNT162b2, AZD1222, or Ad5-nCoV Covid-19 vaccines between February 2 and November 10, 2021, or not

receiving any Covid-19 vaccination. We excluded participants with a probable or confirmed SARS-CoV-2 infection by reverse-transcription polymerase-chain-reaction (RT-PCR) or antigen test before the beginning of the follow-up on February 2 (inclusive), 2021, and individuals who had received at least one dose of a Covid-19 vaccine before that date.

All persons aged 16 years or older are eligible to receive a Covid-19 vaccine. Estimates of the effectiveness of CoronaVac for February 2 through May 1, 2021, including a description of vaccination rollout, the Chilean healthcare system, and vaccine security profile, are available elsewhere.[26] Here, we focus on the effectiveness of third dose homologous and heterologous booster shots for individuals who completed their primary immunization schedule with CoronaVac. On August 11, 2021, the Ministry of Health began administering a booster dose based on a publicly available national vaccination schedule (Supplementary Material). By program indication, individuals aged 55 years or more received one standard dose of AZD1222, and those below 55 years old received one dose of BNT162b2. An alternative booster of CoronaVac was available for all age groups. In our analysis, we classified the cohort participants into three groups: unvaccinated individuals, vaccinated with two CoronaVac doses (≥14 days after receipt of the second vaccine dose and before the third dose), and vaccinated with three doses (≥14 days after receipt of the third vaccine dose using a homologous regimen with CoronaVac, or a heterologous booster with either AZD1222 or BNT162b2).

The study team was entirely responsible for the study design, data collection, and analysis. The authors vouch for the accuracy and completeness of the data. The first, second, and last authors wrote the first draft of the manuscript.

Outcomes and covariates

We estimated the vaccine effectiveness of booster shots using four primary outcomes of interest: laboratory-confirmed Covid-19 cases, hospitalization, admission to the ICU, and death. We considered the time from the beginning of the follow-up, on February 2, 2021, to the onset of symptoms as the endpoint for the four outcomes. Vaccine effectiveness estimates to Covid-19 cases include the more severe outcomes that follow it. The Ministry of Health requires that all suspected Covid-19 cases are notified to health authorities through an online platform and undergo confirmatory laboratory testing. We defined Covid-19 cases and deaths as laboratory-confirmed infections

(92% RT-PCR and 8% antigen test) and corresponding code U07·1 in the International Classification of Diseases 10th revision.

Our analysis included several individual characteristics associated with the probability of vaccination, including booster shots and infection or severity of Covid-19 outcomes. These variables included age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19 illness. These underlying conditions include chronic kidney disease, diabetes, cardiovascular disease, stroke, chronic obstructive pulmonary disease, hematological disease, autoimmune disease, HIV, and Alzheimer's and other dementias.[26]

Analysis strategy

We determined vaccine effectiveness by estimating the hazard ratio between the treated (three doses) and non-treated (unvaccinated) individuals, based on the observed time-to-onset of symptoms, from February 2, 2021, until November 10, 2021. To estimate hazard ratios, we used an extension of the Cox hazards model that allowed accounting for the time-varying vaccination status of participants.[26,27] We adjusted for differences in observed individual characteristics by inverse probability of treatment weighting as in marginal structural models,[28] estimating the weights non-parametrically based on observed characteristics.[29] To account for the time-varying vaccination status and show that our results do not hinge on model specification, we report estimates of the hazard ratios adjusted for age, sex, region of residence, nationality, income, and underlying conditions under both standard and stratified versions of the Cox hazards model, stratifying by all variables in Table S1, Supplementary Material.[26,27]

We estimated the vaccine effectiveness as one minus the corresponding hazard ratio (expressed as a percentage). We show the results for the standard and stratified versions of the Cox hazards model using inverse probability of treatment weighting and without weighting as a robustness check. Inference was based on a partial likelihood approach. It is important to clarify that the comparison of the risk of an event for individuals who received a booster shot and those who were unvaccinated is made at the same calendar time. Each term in the partial likelihood of the effectiveness regression coefficient corresponds to the conditional probability of an individual to express the

outcome of interest from the risk set at a given calendar time. We used the survival package for R version 4.0.5 (Supplementary Material).

Role of the funding source

The funders of this study had no role in the study design, in the collection, analysis, and interpretation of data, in the writing of this manuscript or in the decision to submit the paper for publication.

Results

Study population

Our study cohort included 11,806,589 individuals 16 years or older and affiliated to FONASA, of whom 11,174,257 were eligible. As of November 10, 2021, 1,071,998 participants remained unvaccinated, 678,341 were vaccinated with one Covid-19 vaccine dose, and 9,423,928 completed their primary immunization against Covid-19. Among this group, 7,016,865 (74·5%) participants received two doses of CoronaVac separated by 28 days. Of these, 186,946 received a third dose of CoronaVac, 2,019,260 received a BNT162b2 booster dose, and 1,921,340 received the AZD1222 booster dose, as of November 10, 2021. Table 1 shows descriptive statistics for the study cohort. We found statistically significant differences ($p<0·001$) between Covid-19 patients and the vaccinated and unvaccinated groups by sex, age group, comorbidities, nationality, region of residence, and income. Figure 1 shows the flow diagram of our study cohort.

Effectiveness of vaccination boosters

The Ministry of Health has administered 4,127,546 booster shots during the study period to subjects with a complete primary immunization schedule using CoronaVac. The large majority (95·4%) have been heterologous booster shots, with 46·5% (n=1,921,340) and 48·9% (n=2,019,260) of participants receiving an AZD1222 and BNT162b2 booster, respectively. Only 4·6% (n=186,946) of participants received a homologous booster with CoronaVac.

Based on the weighted stratified version of the Cox model (Table 2, last column), the adjusted vaccine effectiveness against Covid-19 was 78·8% (95% confidence interval, CI, 76·8 to 80·6) for a homologous booster with CoronaVac, 96·5% (95% CI, 96·2 to 96·7) for BNT162b2 booster, and 93·2% (95% CI, 92·9 to 93·6) for the AZD1222 booster.

Additionally, the adjusted vaccine effectiveness against hospitalization was 86·3% (95% CI, 83·7 to 88·5) for a three-dose schedule with CoronaVac, 96·1% (95% CI, 95·3 to 96·9) for BNT162b2 booster, and 97·7 (95% CI, 97·3 to 98·0) for the AZD1222 booster. The adjusted vaccine effectiveness against ICU admissions was 92·2% (95% CI, 88·7 to 94·6) for a three-dose schedule with CoronaVac, 96·2% (95% CI, 94·6 to 97·3) for BNT162b2 booster, and 98·9 (95% CI, 98·5 to 99·2) for the AZD1222 booster. Last, the adjusted vaccine effectiveness against Covid-19 related death was 86·7% (95% CI, 80·5 to 91·0) for a three-dose schedule with CoronaVac, 96·8% (95% CI, 93·9 to 98·3) for BNT162b2 booster, and 98·1% (95% CI, 97·3 to 98·6) for the AZD1222 booster.

Discussion

Our findings show high effectiveness for a homologous booster schedule with CoronaVac and heterologous boosters using AZD1222 or BNT162b2 Covid-19 vaccines. Specifically, the adjusted vaccine effectiveness for a homologous booster with CoronaVac was 78·8% for Covid-19, 86·3% for hospitalization, 92·2% for admission to ICU, and 86·7% for death. The adjusted vaccine effectiveness for individuals with a heterologous BNT162b2 booster shot was 96·5%, 96·1%, 96·2%, and 96·8% for Covid-19, hospitalization, admission to ICU, and death. Last, effectiveness with an AZD1222 booster shot was 93·2%, 97·7%, 98·9%, and 98·1% for Covid-19, hospitalization, admission to ICU, and death. These results exceed the effectiveness of the two-dose primary immunization regimen of CoronaVac previously reported by our group,[26] suggesting that primary immunization with inactivated vaccines should consider a three-dose schedule.

Four studies in Israel have examined the effectiveness of a third dose of BNT162b2 compared to the primary vaccination series. One study estimated a 70-84% reduction in the probability of testing positive for SARS-CoV-2 infection among individuals with a third BNT162b2 dose but did not examine other clinical outcomes.[20] Another study found that the rate of infection in the booster group was lower by a factor of 11.3 for confirmed infection and 19.5 for severe illness but did not adjust for clinical confounders.[21] A third study found a 90% lower Covid-19 related mortality among participants with a third vaccine dose.[22] The fourth study found that the effectiveness of the third dose of BNT162b2 is 93% for admission to the hospital, 92% for severe disease, and 81% against Covid-19 related death.[23] A preprint study in the United States found lower vaccine effectiveness against SARS-CoV-2 infection and hospitalization with a third dose of BNT162b2 (46% and 47% respectively) or mRNA-1273 (47% and

50%). Although not directly comparable, our vaccine effectiveness estimates against hospitalization, ICU admission, and Covid-19 related deaths for the third dose of BNT162b2 are higher, probably because our comparison group is unvaccinated individuals instead of individuals with a complete primary vaccination series.

CoronaVac and Sinopharm's BBIBP-CorV vaccines account for about half the Covid-19 vaccine doses delivered globally and have been administered in 110 primarily low- and middle-income countries.[18] Our results suggest that a three-dose vaccination schedule for CoronaVac, the most commonly used Covid-19 vaccine globally,[18] substantially increases protection against severe illness. However, protection is significantly higher for individuals who received a heterologous vaccine booster compared to a homologous booster with CoronaVac. Our findings may be critical for policymakers, particularly in low-resource settings. Our results for heterologous vaccine booster schedules using BNT162b2 and AZD1222 booster shots also show encouraging results for individuals with a complete primary immunization schedule with CoronaVac, providing additional support for using a mix and match approach.

There is an ongoing global debate about the use of booster shots. Preliminary evidence suggests that the effectiveness of Covid-19 vaccines wanes over time,[6,16,17] although there is no closure on how quick and whether protection against severe Covid-19 also decreases. While the priority should be to ensure that vulnerable individuals across the globe are vaccinated, particularly considering that some Covid-19 vaccines probably provide enough protection against severe disease for the most prevalent SARS-CoV-2 lineages,[30] our results suggest that booster shots substantially increase vaccine effectiveness for CoronaVac. These results are consistent with evidence for BNT162b2 in Israel.[20-23] Rolling out booster Covid-19 vaccine shots parallel to primary immunization campaigns may become a powerful strategy to reduce SARS-CoV-2 infections and mitigate its consequences. These results are aligned with WHO's Strategic Advisory Group of Experts (SAGE) recommendation of providing a third dose to persons aged 60 or older,[31] without neglecting the primary immunization coverage.

The main strengths of our study include the use of a rich cohort of 11·2 million individuals, combining vaccination and administrative healthcare data representing about 80% of the Chilean population. Our data includes demographic variables, residence, income, nationality, and comorbidities, in addition to data on testing, healthcare use, vital statistics, and vaccination. The large sample size allowed us to non-parametrically estimate inverse probability of treatment weights and fit a stratified extended Cox proportional hazards model for the different outcomes of interest (each combination of predictors has a specific hazard function), adding robustness to our

statistical approach. In addition, we assessed the performance of homologous and heterologous booster shots, which provides valuable evidence to policymakers globally, particularly for countries that have used CoronaVac and are considering booster shots. The availability of a diverse matrix of highly effective booster alternatives bypasses potential vaccine supply shortages, helping countries implement and sustain Covid-19 vaccination efforts over time.

The study also has limitations. First, as an observational study, our results may be subject to selection and misclassification biases. There may be selection bias if, for example, vaccinated and unvaccinated individuals have systematic differences. We adjusted our estimates for known confounders that could affect vaccine effectiveness estimates, such as age, sex, region of residence, income, nationality, and whether the subject had underlying conditions associated with severe Covid-19. While our model adjusted for known confounders that could affect the probability of getting vaccinated, infected, or developing severe Covid-19, we cannot account for potentially systematic, unobservable behavioral or health differences between the study groups. For example, publicly reported effectiveness results for the CoronaVac vaccine in Chile during May 2021 may have resulted in fully-vaccinated people taking more risks for acquiring SARS-CoV-2 infection than unvaccinated individuals.[26] As described in the Supplementary Material, the Ministry of Health organized a vaccination rollout through a publicly available vaccination schedule. Individuals need to show up at the nearest vaccination site with an ID; no appointments are required. The risk for misclassification bias on the exposure or the outcomes is low. Chile has a single, electronic, and centralized immunization registry, and SARS-CoV-2 testing is free and widely available.

Second, the Ministry of Health incorporated SARS-CoV-2 genomic surveillance and reported the circulation of four variants of concern (Alfa, Beta, Gamma, and Delta) which may impact vaccine effectiveness (Supplementary Material, section S1·4). Research suggests that Covid-19 vaccines have lower effectiveness against Delta,[5,6] the predominant circulating variant in Chile at the time the study was conducted. We lack representative data to estimate the true prevalence of these variants and their impact on vaccine effectiveness, which could be very relevant to control the pandemic. Third, individuals younger than 55 years were not eligible for AZD1222 booster doses, so results cannot be directly compared among the different vaccines.

Overall, our results suggest that a homologous or heterologous booster vaccine shot for individuals with a complete primary vaccination schedule with CoronaVac results in a high level of protection against Covid-19, including

severe disease and death. However, heterologous boosters showed higher vaccine effectiveness for all outcomes, providing additional support for using a mix and match approach.

Acknowledgments

We thank members of the World Health Organization's Extraordinary Strategic Advisory Group of Experts (SAGE) for suggestions and comments, particularly to Drs. Alejandro Craviotto (Universidad Nacional Autónoma de México), Annelies Wilder-Smith (London School of Hygiene and Tropical Medicine), Daniel Feikin (Johns Hopkins University), and Minal Patel (World Health Organization), and from the Chilean Covid-19 Advisory Group Drs. Catterina Ferreccio (P. Universidad Católica de Chile), Ximena Aguilera (Universidad del Desarrollo), Maria T. Valenzuela (Universidad de Los Andes), Pablo Vial (Universidad del Desarrollo), Gonzalo Valdivia (Pontificia Universidad Católica de Chile), Fernando Otaiza (Ministerio de Salud), and Alvaro Erazo (Pontificia Universidad Católica de Chile) for advice on study design and interpretation of results.

Funding

This research was supported by the Agencia Nacional de Investigación y Desarrollo (ANID) Millennium Science Initiative Program MIDAS [NCN17_059] to AJ; Advanced Center for Chronic Diseases (ACCDiS) ANID FONDAP [15130011] to RA; and Research Center for Integrated Disaster Risk Management (CIGIDEN) ANID FONDAP [15110017] to EU.

Declaration of interests

The authors declare no conflicts of interest.

Data sharing statement

Owing to data privacy laws and regulations in Chile, this study's individual-level data used in this study cannot be shared (Law N19·628). Aggregate data on vaccination and Covid-19 incidence are publicly available at https://github.com/MinCiencia/Datos-COVID19/.

Ethics statement

The research protocol was approved by the Comité Ético Científico Clínica Alemana Universidad del Desarrollo. The study was considered exempt from informed consent, no human health risks were identified. Research analysts belong to the Chilean Ministry of Health; our use of data follows Chilean law 19·628 on personal data protection.

References

1. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *The Lancet Infectious Diseases* 2020; **20**(5): P533-P4.

2. Ledford H, Cyranoski D, Noorden RV. The UK has approved a COVID vaccine — here's what scientists now want to know. *Nature* 2020; **588**: 205-6.

3. Zimmer C, Corum J, Wee S-L. Coronavirus Vaccine Tracker. 2021. https://nyti.ms/3nvAEc4 (accessed November 15 2021).

4. Mathieu E, Ritchie H, Ortiz-Ospina E, et al. A global database of COVID-19 vaccinations. *Nature Human Behaviour* 2021; **5**(7): 947-53.

5. Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of Covid-19 vaccines against the B. 1.617. 2 (Delta) variant. *N Engl J Med* 2021: 585-94.

6. Pouwels KB, Pritchard E, Matthews PC, et al. Effect of Delta variant on viral burden and vaccine effectiveness against new SARS-CoV-2 infections in the UK. *Nature Medicine* 2021; **27**: 2127-35.

7. Feng S, Phillips DJ, White T, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nature Medicine* 2021; **27**: 2032-40.

8. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nature Medicine* 2021.

9. Sauré D, O'Ryan M, Torres JP, Zuniga M, Santelices E, Basso LJ. Dynamic IgG seropositivity after rollout of CoronaVac and BNT162b2 COVID-19 vaccines in Chile: a sentinel surveillance study. *The Lancet Infectious Diseases* 2021: Sept 9, online first.

10. Levin EG, Lustig Y, Cohen C, et al. Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months. *New England Journal of Medicine* 2021; **385**: e84.

11. Mok CKP, Cohen CA, Cheng SMS, et al. Comparison of the immunogenicity of BNT162b2 and CoronaVac COVID-19 vaccines in Hong Kong. *Respirology* 2021: online first.

12. Zeng G, Wu Q, Pan H, et al. Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials. *The Lancet Infectious Diseases* 2021: online first.

13. Thomas SJ, Moreira ED, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine through 6 Months. *New England Journal of Medicine* 2021; **385**: 1761-73.

14. Bergwerk M, Gonen T, Lustig Y, et al. Covid-19 Breakthrough Infections in Vaccinated Health Care Workers. *New England Journal of Medicine* 2021; **385**(16): 1474-84.

15. Fowlkes A, Gaglani M, Groover K, et al. Effectiveness of COVID-19 vaccines in preventing SARS-CoV-2 infection among frontline workers before and during B. 1.617. 2 (Delta) variant predominance—eight US locations, December 2020–August 2021. *Morbidity and Mortality Weekly Report* 2021; **70**(34): 1167.

16. Goldberg Y, Mandel M, Bar-On YM, et al. Waning Immunity after the BNT162b2 Vaccine in Israel. *New England Journal of Medicine* 2021; **385**(24): e85.

17. Feikin D, Higdon MM, Abu-Raddad LJ, et al. Duration of Effectiveness of Vaccines Against SARS-CoV-2 Infection and COVID-19 Disease: Results of a Systematic Review and Meta-Regression. *SSRN Preprint* 2021: https://ssrn.com/abstract=3961378.

18. Mallapaty S. China's COVID vaccines have been crucial — now immunity is waning. *Nature* 2021: 14 October

19. Mahase E. Covid-19 booster vaccines: What we know and who's doing what. *BMJ* 2021; **374**: n2082.

20. Patalon T, Gazit S, Pitzer VE, Prunas O, Warren JL, Weinberger DM. Odds of Testing Positive for SARS-CoV-2 Following Receipt of 3 vs 2 Doses of the BNT162b2 mRNA Vaccine. *JAMA Internal Medicine* 2021.

21. Bar-On YM, Goldberg Y, Mandel M, et al. Protection of BNT162b2 Vaccine Booster against Covid-19 in Israel. *New England Journal of Medicine* 2021; **385**(15): 1393-400.

22. Arbel R, Hammerman A, Sergienko R, et al. BNT162b2 Vaccine Booster and Mortality Due to Covid-19. *New England Journal of Medicine* 2021: online first.

23. Barda N, Dagan N, Cohen C, et al. Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for preventing severe outcomes in Israel: an observational study. *The Lancet* 2021; **398**(10316): 2093-100.

24. Sharma A, Oda G, Holodniy M. Effectiveness of a third dose of BNT162b2 or mRNA-1273 vaccine for preventing post-vaccination COVID-19 infection: an observational study. *medRxiv* 2021: 2021.11.29.21266777.

25. Ministerio de Salud de Chile. Plan Nacional de Vacunación COVID-19 Yo Me Vacuno. 2021. https://www.gob.cl/yomevacuno/ (accessed November 15 2021).

26. Jara A, Undurraga EA, González C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *New England Journal of Medicine* 2021; **385**: 875-84.

27. Cox DR. Regression models and life-tables. *Journal of the Royal Statistical Society: Series B (Methodological)* 1972; **34**(2): 187-202.

28. Robins JM, Hernan MA, Brumback B. Marginal structural models and causal inference in epidemiology. *Epidemiology* 2000; **11**(5): 550-60.

29. Cole SR, Hernán MA. Constructing inverse probability weights for marginal structural models. *American journal of epidemiology* 2008; **168**(6): 656-64.

30. Altmann DM, Boyton RJ, Beale R. Immunity to SARS-CoV-2 variants of concern. *Science* 2021; **371**(6534): 1103-4.

31. Strategic Advisory Group of Experts. Highlights from the Meeting of the Strategic Advisory Group of Experts (SAGE) on Immunization 4-7 October 2021 World Health Organization,, 2021.

Table 1. Characteristics of the study cohort of FONASA affiliates, overall, with laboratory-confirmed Covid-19, and proportion receiving one or more doses of Covid-19 vaccines, February 2 – November 10, 2021*

							Vaccinated					
			COVID-19		Unvaccinated		One dose		Two doses		Three doses	
Characteristic	No.	Col.%	No.	Row%	No.	Row%	No.	Row%	No.	Row%	No.	Row%
Total	11,174,257	100·0	534,314	4·8	1,071,988	9·6	678,341	6·1	4,754,198	42·6	4,669,390	41·8
Sex												
Female	5,993,736	54·0	292,040	4·9	505,607	8·4	298,962	5·0	2,432,234	40·6	2,756,933	46·0
Male	5,180,521	46·0	242,274	4·7	566,381	10·9	379,379	7·3	2,322,304	44·8	1,912,457	36·9
Age group												
16-19	736,905	6·6	32,034	4·3	62,457	8·5	127,731	17·3	514,370	69·8	32,347	4·4
20-29	2,121,616	19·0	127,418	6·0	241,502	11·4	238,566	11·2	1,318,987	62·2	322,561	15·2
30-39	2,001,611	18·0	116,321	5·8	243,211	12·2	144,776	7·2	1,059,332	52·9	554,292	27·7
40-49	1,735,067	16·0	88,973	5·1	165,463	9·5	84,411	4·9	769,601	44·4	715,592	41·2
50-59	1,795,580	16·0	79,308	4·4	136,770	7·6	51,346	2·9	548,956	30·6	1,058,508	59·0
60-69	1,421,931	13·0	49,711	3·5	97·548	6·9	15,666	1·1	289,219	20·3	1,019,498	71·7
70-79	881,220	7·9	26,116	3·0	65,071	7·4	8,657	1·0	148,063	16·8	659,429	74·8
80-more	480,327	4·3	14,433	3·0	59,966	7·2	7,188	1·5	106,010	22·1	307,163	64·0
Comorbidities†												
None	7,586,853	68·0	361,575	4·8	839,456	11·0	558,203	7·4	3,611,727	47·6	2,584,467	34·1
≥ 1	3,587,404	32·0	172,739	4·8	239,532	6·7	120,138	3·4	1,142,811	31·9	2,084,923	58·1
Nationality												
Chilean	10,427,613	93·3	501,394	4·8	895,370	8·6	616,986	5·9	4,417,917	42·4	4,497,340	43·1
Non-Chilean	746,644	6·7	32,920	4·4	176,618	23·7	61,355	8·2	336,621	45·1	172,050	23·0

***Covid-19 denotes coronavirus disease 2019. The study cohort included eligible persons affiliated with the Fondo Nacional de Salud (FONASA), the national public health insurance program which collects, manages, and distributes funds for the public healthcare system in Chile. The model also included individual-level income, and location (16 regions). We found statistically significant differences ($p<0·001$) between Covid-19 patients and the vaccinated and unvaccinated groups by sex, age group, comorbidities, nationality, region of residence, and income. Additional details in Table S1. Covid-19 vaccines include AZD1222, Ad5-nCov, BNT162b2, and CoronaVac (Table 2).

†Coexisting conditions included chronic kidney disease, diabetes, cardiovascular disease (hypertension, myocardial infarction), stroke, chronic obstructive pulmonary disease, hematological disease (lymphoma, leukemia, myeloma), autoimmune disease (rheumatoid arthritis, juvenile idiopathic arthritis, systemic lupus erythematosus), HIV, and Alzheimer's and other dementias.

Table 2. Effectiveness of Covid-19 vaccine CoronaVac, BNT162b2, and AZD1222 boosters in preventing Covid-19 outcomes among cohort participants according to immunization status, February 2–November 10, 2021*

Immunization status	Person-days	Cases		Vaccine effectiveness (95% CI)			
		No.	Incidence rate 1000 person-days	Unweighted, adjusted for all covariates†	Weighted, adjusted for all covariates†	Unweighted, stratified analysis‡	Weighted, stratified analysis‡
Covid-19							
Unvaccinated	1,079,861,007	314,862	0·2916	–	–	–	–
CoronaVac booster (≥14 days after 3 dose)	8,795,237	323	0·0367	75·6 (72·7–78·1)	78·1 (76·1–79·9)	77·3 (74·6–79·8)	78·8 (76·8–80·6)
BNT162b2 booster (≥14 days after 3 dose)	34,755,396	334	0·0096	95·6 (95·1–96·1)	96·3 (96·1–96·5)	95·8 (95·3–96·2)	96·5 (96·2–96·7)
AZD1222 booster (≥14 days after 3 dose)	96,601,030	969	0·0100	92·8 (92·4–93·3)	93·2 (92·8–93·5)	93·1 (92·6–93·5)	93·2 (92·9–93·6)
Hospitalization							
Unvaccinated	1,101,483,596	34,494	0·0313	–		–	–
CoronaVac booster (≥14 days after 3 dose)	8,999,341	89	0·0099	83·4 (79·5–86·6)	84·7 (81·8–87·1)	87·2 (84·1–89·7)	86·3 (83·7–88·5)
BNT162b2 booster (≥14 days after 3 dose)	35,941,136	55	0·0015	95·3 (93·8–96·4)	96·4 (95·6–97·0)	95·7 (94·4–96·7)	96·1 (95·3–96·9)
AZD1222 booster (≥14 days after 3 dose)	98,599,509	139	0·0014	97·3 (96·7–97·7)	97·5 (97·1–97·8)	97·8 (97·4–98·2)	97·7 (97·3–98·0)
Admission to ICU							
Unvaccinated	1,103,499,541	12,343	0·0112	–	–	–	–
CoronaVac booster (≥14 days after 3 dose)	9,046,214	21	0·0023	89·4 (83·6–93·1)	91·1 (87·1–93·9)	92·5 (88·3–95·2)	92·2 (88·7–94·6)
BNT162b2 booster (≥14 days after 3 dose)	36,034,118	16	0·0004	96·1 (93·7–97·7)	96·3 (94·8–97·4)	96·6 (94·4–98·0)	96·2 (94·6–97·3)
AZD1222 booster (≥14 days after 3 dose)	98,801,374	26	0·0003	98·6 (98·0–99·1)	98·8 (98·3–99·1)	99·0 (98·5–99·3)	98·9 (98·5–99·2)
Confirmed death							
Unvaccinated	1,103,668,150	6,367	0·0058	–	–	–	–
CoronaVac booster (≥14 days after 3 dose)	9,059,669	18	0·0020	85·8 (77·2–91·2)	83·7 (76·6–88·7)	88·9 (82·1–93·2)	86·7 (80·5–91·0)
BNT162b2 booster (≥14 days after 3 dose)	36,060,324	6	0·0002	96·8 (92·7–98·5)	96·4 (93·2–98·0)	97·3 (93·9–98·8)	96·8 (93·9–98·3)
AZD1222 booster (≥14 days after 3 dose)	98,845,182	22	0·0002	98·0 (96·4–98·7)	97·9 (97·0–98·5)	98·4 (97·5–98·9)	98.1 (97.3–98.6)

*Participants were classified into three groups: those who were unvaccinated, those who were fully immunized (≥14 days after receipt of the second dose), and those who were vaccinated with CoronaVac and received a booster shot. The 13 days between vaccine administration and partial or full immunization were excluded from the at-risk person-time. We show the results for the

standard and stratified versions of the Cox hazards model using inverse probability of treatment weighting and also without weighting as a robustness check. Covid-19 denotes coronavirus disease 2019, CI denotes confidence intervals.

† The analysis was adjusted for age, sex, 16 regions of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19.

‡ A stratified version of the extended Cox proportional-hazards model was fit to test the robustness of the estimates to model assumptions, stratifying by age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19, and coded as described in Table 1.

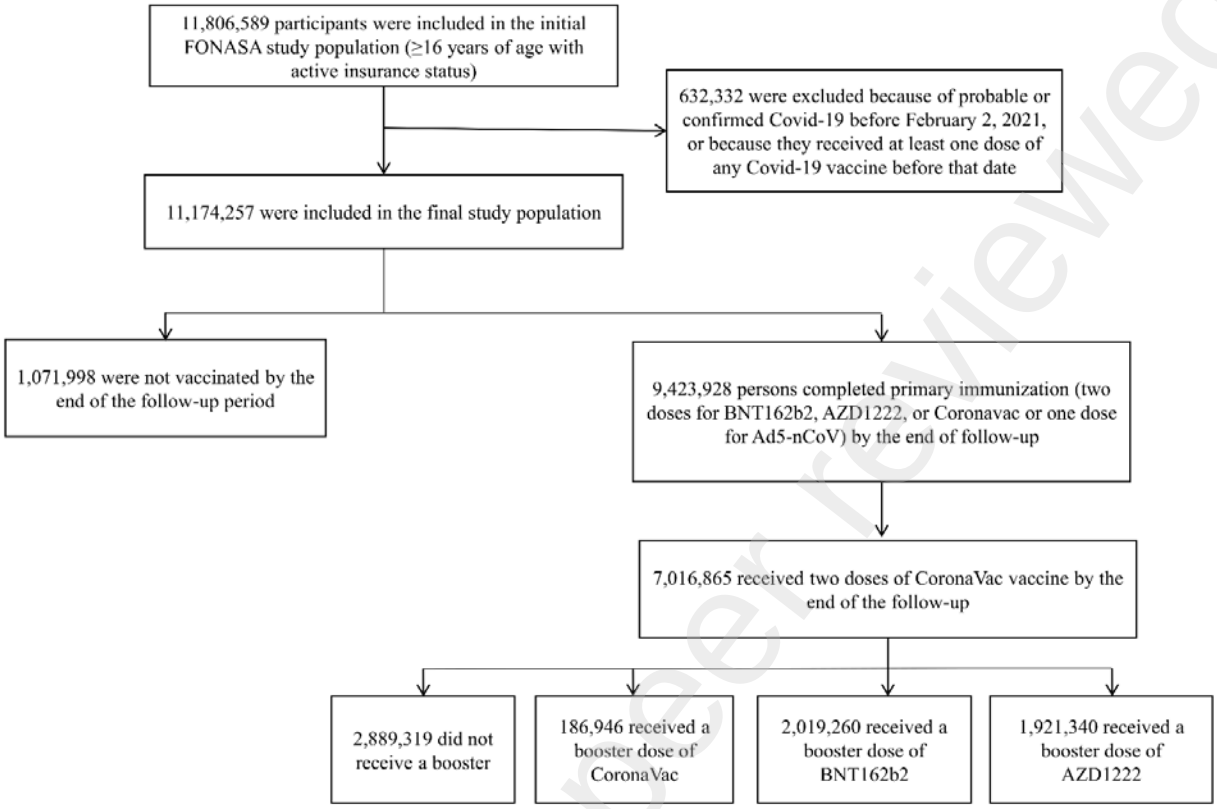


Figure 1. Study participants and cohort eligibility, February 2 to November 10, 2021. Participants were ≥16 years of age, affiliated to the Fondo Nacional de Salud (FONASA), the public national healthcare system, and vaccinated with CoronaVac, BNT162b2, AZD1222, or Ad5-nCoV Covid-19 vaccines between February 2 and November 10, 2021, or not receiving any Covid-19 vaccination. We excluded individuals who had probable or confirmed coronavirus disease 2019 (Covid-19) according to reverse-transcription polymerase-chain-reaction assay for SARS-Cov-2 or antigen test before February 2 (inclusive), 2021.

CoronaVac

O que a ciência comprova

7.6. Dose de reforço da CoronaVac administrada oito meses após a segunda dose aumenta em até cinco vezes os níveis de anticorpos neutralizantes

Uma pesquisa publicada na revista médica britânica The Lancet Infectious Diseases mostrou que a dose de reforço da CoronaVac, vacina do Butantan e da Sinovac, é capaz de aumentar de três a cinco vezes a produção de anticorpos neutralizantes em indivíduos adultos, incluindo idosos com mais de 60 anos. O estudo foi conduzido por pesquisadores chineses da Universidade Fudan, da Sinovac e dos Centros de Controle e Prevenção de Doenças de Nanquim e Hebei.

Na primeira análise, 271 participantes com idades entre 18 e 59 anos imunizados com a CoronaVac receberam a dose de reforço oito meses após a segunda dose, resultando em um aumento de três a cinco vezes nos títulos de anticorpos neutralizantes (NAb) contra o SARS-CoV-2 em comparação com os títulos de anticorpos neutralizantes após a segunda dose.

Um segundo levantamento feito entre 303 adultos com 60 anos ou mais, que também receberam a dose de reforço oito meses após a segunda dose, mostrou que as concentrações de anticorpos neutralizantes aumentaram de 42,9 GMT (ou títulos médios geométricos) no dia 28 após a segunda dose para 158,5 GMT no dia 28 após a dose de reforço – um aumento de 3,7 vezes.

De acordo com os pesquisadores, "nosso estudo descobriu que um esquema de duas doses de CoronaVac gerou boa memória imunológica. A dose de reforço administrada oito meses após a segunda dose foi altamente eficaz em relembrar uma resposta imune específica de SARS-CoV-2, levando a um aumento significativo nos níveis de anticorpos".

Além disso, a pesquisa indica que uma dose de reforço homóloga (com a mesma vacina) pode fornecer imunidade de longa duração e níveis elevados de proteção.

Publicado em: 8/12/2021

Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials

Gang Zeng, Qianhui Wu*, Hongxing Pan*, Minjie Li*, Juan Yang*, Lin Wang, Zhiwei Wu, Deyu Jiang, Xiaowei Deng, Kai Chu, Wen Zheng, Lei Wang, Wanying Lu, Bihua Han, Yuliang Zhao†, Fengcai Zhu†, Hongjie Yu†, Weidong Yin†*

Summary

Background Large-scale vaccination against COVID-19 is being implemented in many countries with CoronaVac, an inactivated vaccine. We aimed to assess the immune persistence of a two-dose schedule of CoronaVac, and the immunogenicity and safety of a third dose of CoronaVac, in healthy adults aged 18 years and older.

Methods In the first of two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials, adults aged 18–59 years in Jiangsu, China, were initially allocated (1:1) into two vaccination schedule cohorts: a day 0 and day 14 vaccination cohort (cohort 1) and a day 0 and day 28 vaccination cohort (cohort 2); each cohort was randomly assigned (2:2:1) to either a 3 μg dose or 6 μg dose of CoronaVac or a placebo group. Following a protocol amendment on Dec 25, 2020, half of the participants in each cohort were allocated to receive an additional dose 28 days (window period 30 days) after the second dose, and the other half were allocated to receive a third dose 6 months (window period 60 days) after the second dose. In the other phase 2 trial, in Hebei, China, participants aged 60 years and older were assigned sequentially to receive three injections of either 1·5 μg, 3 μg, or 6 μg of vaccine or placebo, administered 28 days apart for the first two doses and 6 months (window period 90 days) apart for doses two and three. The main outcomes of the study were geometric mean titres (GMTs), geometric mean increases (GMIs), and seropositivity of neutralising antibody to SARS-CoV-2 (virus strain SARS-CoV-2/human/CHN/CN1/2020, GenBank accession number MT407649.1), as analysed in the per-protocol population (all participants who completed their assigned third dose). Our reporting is focused on the 3 μg groups, since 3 μg is the licensed formulation. The trials are registered with ClinicalTrials.gov, NCT04352608 and NCT04383574.

Findings 540 (90%) of 600 participants aged 18–59 years were eligible to receive a third dose, of whom 269 (50%) received the primary third dose 2 months after the second dose (cohorts 1a-14d-2m and 2a-28d-2m) and 271 (50%) received a booster dose 8 months after the second dose (cohorts 1b-14d-8m and 2b-28d-8m). In the 3 μg group, neutralising antibody titres induced by the first two doses declined after 6 months to near or below the seropositive cutoff (GMT of 8) for cohort 1b-14d-8m (n=53; GMT 3·9 [95% CI 3·1–5·0]) and for cohort 2b-28d-8m (n=49; 6·8 [5·2–8·8]). When a booster dose was given 8 months after a second dose, GMTs assessed 14 days later increased to 137·9 (95% CI 99·9–190·4) for cohort 1b-14d-8m and 143·1 (110·8–184·7) 28 days later for cohort 2b-28d-8m. GMTs moderately increased following a primary third dose, from 21·8 (95% CI 17·3–27·6) on day 28 after the second dose to 45·8 (35·7–58·9) on day 28 after the third dose in cohort 1a-14d-2m (n=54), and from 38·1 (28·4–51·1) to 49·7 (39·9–61·9) in cohort 2a-28d-2m (n=53). GMTs had decayed to near the positive threshold by 6 months after the third dose: GMT 9·2 (95% CI 7·1–12·0) in cohort 1a-14d-2m and 10·0 (7·3–13·7) in cohort 2a-28d-2m. Similarly, in adults aged 60 years and older who received booster doses (303 [87%] of 350 participants were eligible to receive a third dose), neutralising antibody titres had declined to near or below the seropositive threshold by 6 months after the primary two-dose series. A third dose given 8 months after the second dose significantly increased neutralising antibody concentrations: GMTs increased from 42·9 (95% CI 31·0–59·4) on day 28 after the second dose to 158·5 (96·6–259·2) on day 28 following the third dose (n=29). All adverse reactions reported within 28 days after a third dose were of grade 1 or 2 severity in all vaccination cohorts. There were three serious adverse events (2%) reported by the 150 participants in cohort 1a-14d-2m, four (3%) by 150 participants from cohort 1b-14d-8m, one (1%) by 150 participants in each of cohorts 2a-28d-2m and 2b-28d-8m, and 24 (7%) by 349 participants from cohort 3-28d-8m.

Interpretation A third dose of CoronaVac in adults administered 8 months after a second dose effectively recalled specific immune responses to SARS-CoV-2, which had declined substantially 6 months after two doses of CoronaVac, resulting in a remarkable increase in the concentration of antibodies and indicating that a two-dose schedule generates good immune memory, and a primary third dose given 2 months after the second dose induced slightly higher antibody titres than the primary two doses.

Lancet Infect Dis 2021

Published Online December 7, 2021 https://doi.org/10.1016/S1473-3099(21)00681-2

See Online/Comment https://doi.org/10.1016/S1473-3099(21)00696-4

For the Mandarin translation of the abstract see Online for appendix 1

*Contributed equally

†Joint supervisors

Sinovac Biotech, Beijing, China (G Zeng PhD, Le Wang MSc, W Yin MBA); **School of Public Health, Fudan University, Key Laboratory of Public Health Safety, Ministry of Education, Shanghai, China** (Q Wu MPH, J Yang PhD, X Deng MSc, W Zheng BSc, W Lu BSc, Prof H Yu PhD); **Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China** (H Pan MSc, K Chu MSc, Prof F Zhu MD); **Hebei Provincial Center for Disease Control and Prevention, Shijiazhuang, Hebei, China** (M Li MSc, Z Wu MSc, B Han MSc, Prof Y Zhao MSc); **Sinovac Life Sciences, Beijing, China** (Li Wang MSc, D Jiang MSc); **Shanghai Institute of Infectious Disease and Biosecurity, Fudan University, Shanghai, China** (J Yang, Prof H Yu); **Department of Infectious Diseases, Huashan Hospital, Fudan University, Shanghai, China** (Prof H Yu)

Correspondence to: Yuliang Zhao, Hebei Center for Disease Control and Prevention, Shijiazhuang 050021, China **yuliang_zh1@163.com**

or

Fengcai Zhu, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210000, China
jszfc@vip.sina.com

or

Hongjie Yu, School of Public Health, Fudan University, Key Laboratory of Public Health Safety, Ministry of Education, Shanghai 200032, China
yhj@fudan.edu.cn

or

Weidong Yin, Sinovac Biotech, Beijing 100085, China
yinwd@sinovac.comv

Funding National Key Research and Development Program, Beijing Science and Technology Program, and Key Program of the National Natural Science Foundation of China.

Introduction

More than 20 vaccines have been approved for use in response to the COVID-19 pandemic,[1] with over 6·33 billion doses administered globally as of Oct 3, 2021.[2] Following primary vaccination with vaccines including BNT162b2[3–5] (Pfizer–BioNTech's mRNA vaccine), mRNA-1273[4,6] (Moderna's mRNA vaccine), and ChAdOx1 nCoV-19[7,8] (AstraZeneca's non-replicating adenoviral vectored vaccine), neutralising antibody titres and vaccine effectiveness against symptomatic illness have been observed to decrease over time, particularly against the delta (B.1.617.2) variant of SARS-CoV-2, which has become the predominant strain across the globe.[9] A booster dose given 6–8 months after the second dose of BNT162b2,[10] mRNA-1273,[11] and NVX-CoV2373[12] (Novavax's protein subunit vaccine) greatly increased neutralising antibody concentrations, and thus increased neutralisation capacity against the delta variant. Booster vaccination with BNT162b2 was initiated in Israel in response to a surge of COVID-19 cases caused by the delta variant;[13] interim results show that the booster dose significantly reduces rates of confirmed infection and severe illness.[14]

CoronaVac (Sinovac Life Sciences, Beijing, China), an inactivated vaccine against COVID-19, has been authorised for conditional use in China,[15] and is included

Research in context

Evidence before this study

We used the terms "SARS-CoV-2", "COVID-19", "vaccine", and "clinical trial" to search PubMed and Europe PMC on Sept 29, 2021, without language or date restrictions, to identify seven research articles on the immune persistence of currently approved vaccines or the immunogenicity of additional doses in the general population. Previous research reported that neutralising antibody responses elicited by mRNA vaccines (BNT162b2, developed by Pfizer and BioNTech, and mRNA-1273, developed by Moderna), adenovirus-vectored vaccines (ChAdOx1 nCoV-19, developed by Oxford and AstraZeneca, and Ad26.COV2-S, developed by Janssen), an inactivated vaccine (CoronaVac, developed by SinoVac), and a protein subunit vaccine (NVX-CoV2373, developed by Novavax) persisted for 6–8 months after full-schedule vaccination and declined to varying degrees. Neutralising antibodies against variants of concern started at lower concentrations than they did against the original alpha variant and waned substantially, especially against the beta (B.1.351) variant, whereas neutralising antibody concentrations against other variants of concern were less affected. Neutralisation capacity against the delta (B.1.617.2) variant, mediated by a homologous third dose given 6–8 months after the second dose of mRNA-1273, BNT162b2, or ChAdOx1 nCoV-19, increased multifold and was similar to or higher than the level against the ancestral SARS-CoV-2 after the second dose. Several clinical trials have explored heterologous vaccination schedules with ChAdOx1 nCoV-19 and BNT162b2, BNT162b2 and Ad26.COV2-S, CoronaVac and ChAdOx1 nCoV-19, and CoronaVac and Convidecia (adenovirus type-5-vectored vaccine, developed by CanSino), showing that heterologous vaccination can induce robust immune responses in adults aged 18 years and older. These results indicate flexibility in deploying COVID-19 vaccines in mix-and-match schedules.

Added value of this study

Our phase 2 trial among adults aged 18–59 years provides preliminary evidence of 6-month immune persistence after two two-dose schedules (14-day and 28-day intervals) of CoronaVac and immunogenicity and safety of a third dose of CoronaVac given 2 months or 8 months after the second dose. Neutralising antibody titres induced by two doses of CoronaVac (3 μg formulation) declined to near or below the lower limit of seropositivity after 6 months. A third dose given 8 months after the second dose led to a strong boost in immune response (a three-fold to five-fold increase in neutralising antibody titres 28 days after the second dose). Our phase 2 trial in healthy adults aged 60 years and older found that neutralising antibody titres declined to low concentrations 6 months after the second dose but rapidly rebounded after a third dose given at 8 months after the second dose (an approximate three-fold increase in neutralising antibody titre). Seropositivity after an 8-month third dose was 98–100% regardless of age group. No safety concerns were seen with a third dose; reactogenicity of the vaccine was indistinguishable from reactogenicity of aluminium hydroxide placebo. This study provided data on immune persistence after primary immunisation with CoronaVac, and immunogenicity and safety of a third homologous dose in adults aged 18 years or older.

Implications of all the available evidence

The rapid and robust rebound in immunity induced by a third dose of CoronaVac showed that primary vaccination with two doses induced immune memory in adults aged 18 years and older. A third dose was immunogenic and markedly increased neutralising antibody titres when given 8 months after the second dose. Therefore, a third dose might provide additional benefit, including longer-lasting immunity and higher level of protection, over a two-dose schedule, but such determinations need longer-term study and real-world studies of vaccine effectiveness.

in WHO's emergency use listing.[16] This vaccine has been administered in 26 countries, including China,[1] and is increasing the global supply through COVAX.[17] In China, 2·21 billion doses of COVID-19 vaccines have been administered as of Oct 3, 2021,[18] the vast majority of which are inactivated vaccines. Evidence from real-world studies of CoronaVac in two-dose schedules in Chile,[19] Brazil,[20] and China[21,22] shows that the vaccine effectively prevents laboratory-confirmed COVID-19, with greater effectiveness against more severe outcomes, including in settings with circulation of variants of concern. However, persistence of CoronaVac vaccine-induced immunity is unknown, and the immunogenicity and safety of a booster dose has not been determined.

To fill this knowledge gap, we aimed to assess immune persistence after primary immunisation with CoronaVac, and immunogenicity and safety of a third homologous dose, in two population groups: adults aged 18–59 years and adults aged 60 years or older.

Methods

Study design and participants

Our study is built upon two single-centre, double-blind, randomised, placebo-controlled, phase 2 clinical trials of CoronaVac. One trial was initiated in Suining County, Jiangsu province, China, by Jiangsu Provincial Center for Disease Control and Prevention (CDC) on May 3, 2020, among healthy adults aged 18–59 years, and the other was initiated in Renqiu, Hebei province, China, by Hebei Provincial CDC, on June 12, 2020, among healthy adults aged 60 years and older. The designs of the phase 2 trials have been published previously.[23,24] Briefly, key exclusion criteria for trial enrolment included suspected or laboratory-confirmed SARS-CoV-2 infections and known allergy to any vaccine component. A complete list of exclusion criteria is in the protocol (appendix 2 pp 74–76; appendix 3 pp 38–39).

For the trial in adults aged 18–59 years, eligible participants were initially recruited and randomly allocated (1:1) to vaccination cohorts with two-dose schedules, either 14 days apart (cohort 1) or 28 days apart (cohort 2). Within each cohort, participants were randomly allocated (2:2:1) to either a 3 μg group, a 6 μg group, or a placebo group. For the trial in adults aged 60 years and older, eligible participants were assigned (2:2:2:1) sequentially to receive two doses 28 days apart of either 1·5 μg, 3 μg, or 6 μg vaccine or placebo (cohort 3). Randomisation codes for each vaccination schedule cohort were generated individually and randomly assigned using block randomisation developed with SAS version 9.4. Adults aged 18–59 years were assigned with a block size of five and adults aged 60 years and older were assigned with a block size of 14. Concealed random group allocations and blinding codes were kept in signed and sealed envelopes. Investigators, participants, and laboratory staff were masked to group assignment. The randomisation code was assigned to each participant in sequence in the order of enrolment by investigators, who were involved in the rest of the trial.

1·5 μg, 3 μg, or 6 μg doses of CoronaVac (Vero cell, inactivated CN02 strain of SARS-CoV-2 with 1·5, 3, or 6 μg per 0·5 mL of aluminium hydroxide adjuvant) or placebo (0·5 mL of aluminium hydroxide adjuvant) in prefilled syringes were administered by intramuscular injection into the deltoid muscle. To evaluate the immunogenicity of primary vaccination, blood samples were taken before vaccination and at day 28 after the second dose. Interim results of these data have been published.[23,24] For the trial in adults aged 18–59 years, the protocol was amended on Dec 25, 2020, to evaluate the immunogenicity of an additional dose (appendix 2 p 3). The amended protocol was updated on ClinicalTrials.gov. According to the order of the blocks, half of the participants were sequentially allocated to receive an additional dose of the vaccine or placebo at 28 days after the second dose (with a 30-day window period; hereafter cohort 1a-14d-2m and cohort 2a-28d-2m, with 14d and 28d representing the interval in days between the first two doses, and 2m denoting the actual median interval in months between the second and third doses), and the other half were allocated to receive a booster dose 6 months after the second dose (with a 60-day window period; hereafter cohort 1b-14d-8m and cohort 2b-28d-8m, with 8m denoting the actual median interval in months between the second and third doses). For the trial in adults aged 60 years and older, a booster dose was given 6 months after the second dose (with a 90-day window period; cohort 3-28d-8m) per the original protocol (appendix 3 p 41–42). Key exclusion criteria for third doses are shown in appendix 4 (p 3). Written informed consent was obtained from participants both before enrolment and before administration of a third dose of a vaccine in eligible participants. The clinical trial protocol and informed consent forms for the study in adults aged 18–59 years were approved by the Jiangsu Ethics Committee (JSJK2020-A021–02), and those for the study in adults aged 60 years and older were approved by Hebei CDC Ethics Committee (IRB2020–006).

Essential steps and timing for each visit specified in the protocol are shown in appendix 4 (p 4). Participants in each cohort received homologous third doses, vaccine or placebo. Participants were to be withdrawn from the trial if they had an unacceptable adverse event as judged by the investigators and defined by the Guidelines of the National Medical Products Administration for Adverse Event Classification Standards for Clinical Trials of Preventive Vaccines (2019), an unacceptable health status as judged by the investigators, or abnormal clinical manifestations as judged by the investigators, or at the participant's request or for any other reason judged necessary by the investigator. The trial would be suspended under the following conditions as judged by the investigators: occurrence of one or more grade 4 local or systemic adverse reactions related to vaccination or more than 15% of the participants

For more on **the amendment to the NCT04352608 trial** see https://clinicaltrials.gov/ct2/show/NCT04352608

See Online for appendix 4

See Online for appendix 2

See Online for appendix 3

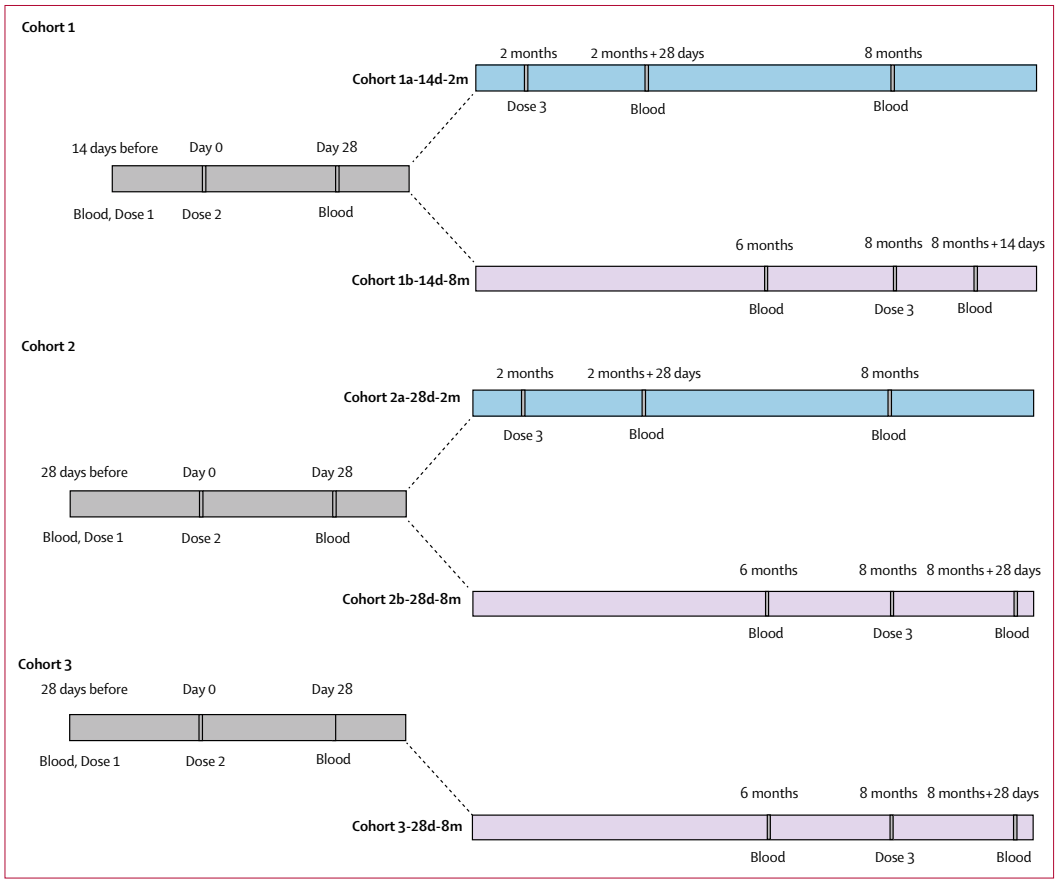


Figure 1: **Trial process timeline**
Blood=blood sample taken.

having grade 3 or above adverse reactions, including local reactions, systemic reactions, and vital sign changes. During the trial periods, no active surveillance for natural infection with SARS-CoV-2 was done by this study. SARS-CoV-2 occurring in study participants was required to be reported to the investigator. Under the China Government's COVID-19 prevention and control policy of zero tolerance for local transmission, all infections are identified in a timely manner and reported by local health departments for contact tracing, isolated treatment, and quarantine of close contacts and testing for SARS-CoV-2 RNA.

For participants who received their third dose 28 days after the second dose (cohort 1a-14d-2m and cohort 2a-28d-2m), blood samples were collected on day 28 and month 6 after the third dose to evaluate immunogenicity and immune persistence of the third dose. For participants who received their third dose 6 months after the second dose (cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m), blood samples were collected at month 6 after the second dose to evaluate the immune persistence of the second dose, and on day 28 after the third dose to assess immunogenicity of the third dose (with the exception of cohort 1b-14d-8m, in which samples were collected on day 14 after the third dose; figure 1; appendix 4 p 4).

Safety information after the third dose was obtained by the same methods as for the first two doses, as described previously.[23] Participants were required to record injection-site adverse events (eg, pain, redness, and swelling), or systemic adverse events (eg, allergic reactions, cough, and fever) on diary cards for 7 days after their third dose. For days 8–28, unsolicited adverse reactions were collected by spontaneous reporting from participants in all cohorts. We planned to collect serious adverse events until 6 months after the third dose for participants in cohorts 1 and 2, and until 1 year after third dose for participants in cohort 3. The cut-off day of this report was 6 months after the second dose for participants in cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m, and 6 months after the third dose for

participants in cohort 1a-14d-2m and cohort 2a-28d-2m. Reported adverse events were graded according to China National Medical Products Administration guidelines.[23] Serious adverse events were coded by the Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class. The existence of causal associations between adverse events and vaccination was determined by the investigators.

Immunological assessment methods and related procedures are described in appendix 4 (p 5). Neutralising antibodies against infectious SARS-CoV-2 (virus strain SARS-CoV-2/human/CHN/CN1/2020, GenBank accession number MT407649.1) were quantified using a microcytopathogenic effect assay.[23] Several measures were taken to control the quality of the microcytopathogenic effect assay, including virus back-titration for each batch of tests to determine whether the amount of virus was within the range of 32–320 tissue culture infectious dose ($TCID_{50}$) per 50 µL.[25] Two types of positive antibody control, a negative antibody control, a serum toxicity control, and a cell control were included for each test. Blood samples taken at baseline and 28 days after the second dose had been tested previously, and the neutralising antibody titres were comparable between the group aged 18–59 years and those aged 60 years or older.[23,24]

Blood samples taken 6 months after the second dose or 14 days, 28 days, or 6 months after the third dose were tested in our analyses. However, neutralising antibody titres of sera obtained on day 28 after the third dose from participants in the older age group were approximately two-fold higher (352·8 [95% CI 266·4–441·1] in cohort 3-28d-8m) than titres from participants in the younger age group (143·1 [95% CI 110·8–184·7] in cohort 2b-28d-8m) who had been immunised with the same vaccination schedule. To verify the stability and reliability of the neutralising antibody test results, we retested a convenient random sample of specimens from 100 adults in the younger age group and 100 adults in the older age group. In the group of younger adults, neutralising antibody titres were consistent between the first test and the retest. Accordingly, the results of the first test were used in our analysis for this population. In the group of older adults, neutralising antibody titres were significantly lower in the retests than they were in the first tests. Considering the acceptable results of serum samples in younger adults and older adults in the retests, and the consistence of our procedures with the protocol after evaluation, we used the retest results of the 100 adults in the older age group, which we believe to be more reliable, in our analyses. Due to repeated freezing and thawing, and insufficient quantity of sera, we were unable to retest specimens from the other adults in the older age group. A detailed description of retest procedures and results for the older adults is provided in appendix 4 (pp 9–11).

Outcomes

The primary immunological outcomes of the two phase 2 trials have been reported previously;[23,24] here, we report the results of prespecified secondary and exploratory immunological outcomes. Secondary immunological outcomes included geometric mean titres (GMTs), geometric mean increases (GMIs), and seropositivity of neutralising antibodies to infectious SARS-CoV-2 28 days after the third dose (for cohort 1a-14d-2m and cohort 2a-28d-2m). Exploratory immunological outcomes included GMTs and seropositivity at 6 months after the second dose (for cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m) and at 14 days (for cohort 1b-14d-8m) or 28 days (for cohort 2b-28d-8m and cohort 3-28d-8m) after the third dose. The additional outcome of GMTs and seropositivity at 6 months after the third dose for cohort 1a-14d-2m and cohort 2a-28d-2m was a post-hoc analysis. To assess the immunogenicity of a third dose, we included the participants who received their assigned third doses and had available antibody results on day 28 after the third dose (day 14 after the third dose for cohort 1b-14d-8m); defined as the per-protocol analysis set of third doses. To assess the immune persistence of primary two-dose series we included participants who completed 6-month follow-up after two doses for cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m; to assess the immune persistence of primary three-dose series we included participants who completed 6-month follow-up after three doses for cohort 1a-14d-2m and cohort 2a-28d-2m; defined as the immune persistence analysis set. We defined seropositivity as a titre of 8 or greater for neutralising antibodies to infectious SARS-CoV-2. Primary safety endpoints included any adverse reactions within 28 days after dose three in all cohorts. Secondary safety endpoints were serious adverse events occurring from the first dose to 6 months after the third dose in all vaccination cohorts. A complete list of outcomes is provided in appendix 4 (pp 6–7). Given that the 3 µg dose is the licensed formulation, and owing to space constraints, we mainly present results for the 3 µg group in the main text and provide detailed results for other intervention groups in tables and appendix 4.

Statistical analysis

The sample size was determined following requirements of the National Medical Products Administration, China's regulatory authority for vaccines. We assessed immunological endpoints in the per-protocol population, which included all participants who completed their assigned third doses and had antibody results available according to the protocol. In addition, we assessed the immune persistence of primary immunisation in the immune-persistence analysis set, which included participants who completed 6-month follow-up after two doses for cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m and who completed 6-month follow-up after three doses for cohort 1a-14d-2m and cohort 2a-28d-2m. Serious adverse

	1·5 µg group	3 µg group	6 µg group	Placebo group
Cohort 1a-14d-2m (a third dose at month 2 after the second dose)				
Number of participants	NA	55	58	26
Age, years	NA	45·2 (9·1)	44·7 (8·6)	44·3 (8·6)
Male	NA	29 (53%)	20 (34%)	10 (38%)
Female	NA	26 (47%)	38 (66%)	16 (62%)
Cohort 1b-14d-8m (a third dose at month 8 after the second dose)				
Number of participants	NA	55	56	30
Age, years	NA	40·4 (10·3)	42·4 (8·8)	44·8 (6·9)
Male	NA	24 (44%)	27 (48%)	12 (40%)
Female	NA	31 (56%)	29 (52%)	18 (60%)
Cohort 2a-28d-2m (a third dose at month 2 after the second dose)				
Number of participants	NA	54	50	26
Age, years	NA	42·5 (8·6)	40·7 (9·4)	44·0 (7·7)
Male	NA	34 (63%)	26 (52%)	14 (54%)
Female	NA	20 (37%)	24 (48%)	12 (46%)
Cohort 2b-28d-8m (a third dose at month 8 after the second dose)				
Number of participants	NA	52	50	28
Age, years	NA	44·3 (9·5)	43·1 (9·9)	45·7 (9·7)
Male	NA	23 (44%)	26 (52%)	11 (39%)
Female	NA	29 (56%)	24 (48%)	17 (61%)
Cohort 3-28d-8m (a third dose at month 8 after the second dose)				
Number of participants	85	90	81	47
Age, years	66·3 (4·4)	66·4 (4·4)	66·3 (4·4)	67·1 (4·7)
Male	41 (48%)	44 (49%)	37 (46%)	27 (57%)
Female	44 (52%)	46 (51%)	44 (54%)	20 (43%)

Data are n (%) or mean (SD). NA=not applicable.

Table 1: **Baseline demographic characteristics in the safety population of participants who received the third dose**

events were evaluated in the safety population, which included all participants who received at least one dose of study vaccine from the beginning of the vaccination schedule. Safety assessments for the third dose were done in a safety population data set of all participants who received a third dose.

The demographics of participants who received the third dose were summarised for vaccination cohorts, and Pearson χ^2 test or Fisher's exact test were used to analyse categorical outcomes. We calculated 95% CIs for all categorical outcomes using the Clopper-Pearson method. We calculated GMTs and corresponding 95% CIs on the basis of the standard normal distribution of log-transformed antibody titres. For the third dose given at 28 days after the second dose (cohort 1a-14d-2m and cohort 2a-28d-2m), GMIs were calculated using antibody titres before vaccination and at 28 days after the third dose (taking prevaccination as baseline). For the booster dose given 6 months after the second dose (cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m), GMIs were calculated using antibody titres before (ie, 6 months after the second dose) and at 28 days or 14 days after the third (booster) dose (taking pre-booster as baseline). ANOVA models with log-transformation (per GMT and GMI as above) were used to detect differences among groups. Post-hoc generalised liner mixed models (GLMM) were done to compare antibody concentrations induced by the third dose among participants in the four groups in cohorts 1 and 2, accounting for age, sex, dose group, vaccine schedule, interactions of dose and schedule, sampling time, and a random intercept for each participant.

Comparisons were done between groups by group t-tests with log-transformation and Bonferroni correction done as a post-hoc test if variance was significant. Hypothesis testing was two-sided, and we considered p values of less than 0·05 to be significant. We used R software version 3.6.0 for all analyses. The clinical trial is supervised by an independent data monitoring committee that consists of an independent statistician, a clinician, and an epidemiologist. Detailed information on the members is provided in appendix 4 (p 8). The trials are registered with ClinicalTrials.gov, NCT04352608 and NCT04383574.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

On May 3, 2020, 600 participants aged 18–59 years were enrolled into the phase 2 trial, of whom 540 (90%) were eligible and allocated to receive third doses (appendix 4 pp 13–14). Of these 540 participants, 139 (26%) participants were allocated to cohort 1a-14d-2m and 130 (24%) participants were allocated to cohort 2a-28d-2m; cohort 1a-14d-2m received a third dose at a median of 2 months (IQR 56–56 days) and cohort 2a-28d-2m received a third dose at a median of 2 months (IQR 51–51 days) after the second dose. 135 (97%) of 139 participants from cohort 1a-14d-2m and 124 (95%) of 130 participants from cohort 2a-28d-2m completed blood sampling to assess immune persistence for 6 months after dose three. Separately, 147 (25%) of the 600 participants assigned to cohort 1b-14d-8m and 138 (23%) assigned to cohort 2b-28d-8m were followed up for 6 months after the second dose, and 141 participants in cohort 1b-14d-8m (26% of the 540 participants eligible for a third dose) and 130 participants in cohort 2b-28d-8m (24% of the 540 participants eligible for a third dose) received a third dose at month 8 after the second dose for immunogenic evaluation (figure 1).

On June 12, 2020, 350 participants aged 60 years and older were enrolled in the phase 2 trial and 303 (87%) were allocated to receive third doses at month 8 after the second dose (appendix 4 p 15). 98 (32%) of the 303 participants were included in the immunogenicity analysis as described in the Methods (two participants were excluded due to protocol violation). The demographic characteristics of these 98 participants were similar to the other participants in the same age group

Figure 2: **Level of neutralising antibodies to infectious SARS-CoV-2 in adults aged 18–59 years**
Dots are reciprocal neutralising antibody titres for individuals in the per-protocol population. Numbers above the bars are GMTs, and the error bars indicate the 95% CI. The dotted horizontal line represents the seropositivity threshold. Titres lower than the limit of detection (1/4) are presented as half the limit of detection. Numbers above the short horizontal lines are p values of comparisons between 3 µg group and 6 µg group. GMT=geometric mean titre.

(appendix 4 pp 9–11). All participants in the older age group were included in the safety analyses.

No natural infections were reported in any cohort. There were 141 minor protocol deviations in cohort 1b-14d-8m, including 141 participants given third doses 9–11 days outside of the prespecified time window, which did not result in exclusion of participants from the analysis (appendix 3 p 12). Mean ages of participants were between 40·4 years (SD 10·3) and 45·7 years (9·7) in cohorts 1 and 2 (adults aged 18–59 years old), and between 66·3 years (SD 4·4) and 67·1 years (4·7) in cohort 3 (adults aged 60 years and older; table 1). At baseline, none of the participants in any cohort had detectable neutralising antibodies (figures 2, 3).

A third dose of CoronaVac given at month 2 after the second dose moderately increased neutralising antibody levels induced by the first two doses. In the 3 µg group, the GMT in cohort 1a-14d-2m on day 28 after dose 2 was 21·8 (95% CI 17·3–27·6) and on day 28 after dose 3 was 45·8 (35·7–58·9), and in cohort 2a-28d-2m GMT on day 28 after dose 2 was 38·1 (95% CI 28·4–51·1) and on day 28 after dose 3 was 49·7 (39·9–61·9; figure 2; table 2). GMIs of neutralising antibodies from baseline to 28 days after the third dose were 22·9 (95% CI 17·8–29·4) for cohort 1a-14d-2m and 24·8 (19·9–31·0) for cohort 2a-28d-2m (table 2). Seropositivity rates in all vaccination groups in cohorts 1a-14d-2m and 2a-28d-2m were above 95% at 28 days after three doses (table 2).

Results of immune persistence analysis from cohort 1a-14d-2m and cohort 2a-28d-2m show that, by 6 months after the third dose, the GMT was approximately 10 and seropositivity remained above 50% (appendix 4 pp 16–17). GMTs in cohort 1a-14d-2m on day 28 (p=0·0053) and at month 6 (p=0·039) after the third dose were significantly higher in the 6 µg group than in the 3 µg group, whereas there was no significant difference between the two doses at either timepoint in cohort 2a-28d-2m (appendix 4 pp 16–17).

Regardless of the interval between the first two doses, neutralising antibody titres declined to below the

	1·5 µg group	3 µg group	6 µg group	Placebo	p value*	p value†
Cohort 1a-14d-2m						
Seropositivity	NA	53/54 (98%; 90·11–99·95)	57/58 (98%; 90·76–99·96)	0/26 (0·00–13·23)	<0·0001	1·00
GMT (95% CI)	NA	45·8 (35·7–58·9)	74·2 (59·0–93·3)	2·0 (2·0–2·0)	<0·0001	0·0053
GMI (95% CI)	NA	22·9 (17·8–29·4)	37·1 (29·5–46·6)	1·0 (1·0–1·0)	<0·0001	0·0052
Cohort 1b-14d-8m‡						
Seropositivity	NA	53/53 (100%; 93·28–100·00)	55/55 (100%; 93·51–100·00)	0/30 (0·00–11·57)	<0·0001	1·00
GMT (95% CI)	NA	137·9 (99·9–190·4)	175·1 (138·8–221·0)	2·0 (2·0–2·0)	<0·0001	0·23
GMI (95% CI)	NA	35·1 (24·3–50·7)	36·9 (28·5–47·8)	1·0 (1·0–1·0)	<0·0001	0·82
Cohort 2a-28d-2m						
Seropositivity	NA	52/53 (98%; 89·93–99·95)	48/48 (100%; 92·60–100·00)	0/25 (0·00–13·72)	<0·0001	1·00
GMT (95% CI)	NA	49·7 (39·9–61·9)	51·9 (41·3–65·3)	2·0 (2·0–2·0)	<0·0001	0·78
GMI (95% CI)	NA	24·8 (19·9–31·0)	26·0 (20·7–32·7)	1·0 (1·0–1·0)	<0·0001	0·78
Cohort 2b-28d-8m						
Seropositivity	NA	49/49 (100%; 92·75–100·00)	48/48 (100%; 92·60–100·00)	0/27 (0·00–12·77)	<0·0001	1·00
GMT (95% CI)	NA	143·1 (110·8–184·7)	215·7 (162·6–286·2)	2·0 (2·0–2·0)	<0·0001	0·03
GMI (95% CI)	NA	21·2 (15·3–29·2)	30·4 (21·5–43·0)	1·0 (1·0–1·0)	<0·0001	0·24
Cohort 3-28d-8m§						
Seropositivity	27/28 (96%; 81·65–99·91)	29/29 (100%; 88·06–100·00)	27/28 (96%; 81·65–99·91)	0/13 (0·00–24·71)	<0·0001	0·49
GMT (95% CI)	99·6 (62·0–159·9)	158·5 (99·0–253·7)	178·9 (125·2–255·6)	2·0 (2·0–2·0)	<0·0001	0·37
GMI (95% CI)	28·2 (16·8–47·4)	39·7 (23·6–66·6)	44·2 (27·2–71·9)	0·9 (0·7–1·1)	<0·0001	0·77

Data are n/N (%; 95% CI) unless otherwise stated. ANOVA model with log-transformation (per GMT and GMI as above) was used to detect the difference among groups. Comparison between groups was conducted by group t-test with log-transformation. GMT=geometric mean titre. GMI=geometric mean increase. NA=not applicable. *p values are for comparisons among all groups. †p values are for comparisons between the 3 µg group and the 6 µg group. ‡Immunogenicity was assessed on day 14 after the third dose. §p values for comparisons between the 1·5 µg group and the 3 µg group were 0·49 for seropositivity, 0·18 for GMTs, and 0·37 for GMIs; p values for comparisons between the 1·5 µg group and the 6 µg group were 1·00 for seropositivity, 0·06 for GMTs, and 0·22 for GMIs.

Table 2: **Immunogenicity assessment on day 28 after the third dose**

seropositive cutoff by 6 months after the second dose (GMT 3·9 [95% CI 3·1–5·0] in cohort 1b-14d-8m and 6·8 [5·2–8·8] in cohort 2b-28d-8m; figure 2). In the immune persistence analysis set, at month 6 after the second dose, ten (17%) of 59 participants in cohort 1b-14d-8m and 19 (35%) of 54 participants in cohort 2b-28d-8m were seropositive (appendix 4 pp 18–19).

In post-hoc analyses, after administering a booster dose at 8 months after the second dose, GMTs increased to 137·9 (95% CI 99·9–190·4) in cohort 1b-14d-8m 14 days later, and to 143·1 (110·8–184·7) in cohort 2b-28d-8m 28 days later (figure 2). Neutralising antibody concentrations 14 days after dose 3 were approximately five-fold higher than neutralising antibody concentrations on day 28 after the second dose in cohort 1b-14d-8m (from a GMT of 27·4 to 137·9 in the 3 µg group and from a GMT of 30·4 to 175·1 in the 6 µg group), and in cohort 2b-28d-8m, neutralising antibody titres 28 days after the third dose were approximately three-fold higher than neutralising antibody titres 28 days after the second dose (from a GMT of 45·9 to 143·1 in the 3 µg group; table 2, figure 2). Seropositivity on day 14 after the third dose in cohort 1b-14d-8m and on day 28 after the third dose in cohort 2b-28d-8m was 100% for both doses (table 2). GMIs from before to after the booster dose were 35·1 (95% CI 24·3–50·7) in cohort 1b-14d-8m and 21·2 (15·3–29·2) in cohort 2b-28d-8m (table 2).

In GLMM models, neutralisation titres decreased with increasing age (appendix 4 p 21). Immune responses induced by 6 µg doses were better than those induced by 3 µg doses, and a third dose significantly raised antibody levels compare with 28 days after dose 2. The vaccination

schedule used in cohort 2b-28d-8m produced the best immunogenicity (appendix 4 p 21).

In the immune persistence analysis of cohort 3-28d-8m, in the 3 µg group, neutralising antibody titres had declined to below the seropositive cutoff at 6 months after the second dose (from 40·8 [95% CI 33·8–49·3] at day 28 after dose 2 to 3·4 [2·9–4·1]), and 17 (18%) of 98 participants were seropositive (appendix 4 p 20). A booster dose given 8 months after the second dose increased the GMT to 158·5 (95% CI 96·9–259·2) 28 days after the booster dose (figure 3, table 2). The GMI from before to after the booster dose was 39·7 (95% CI 23·6–66·6; table 2). GMTs on day 28 after the third dose were highest in the 6 µg group (p<0·0001) and similar between the 3 µg group and the 1·5 µg group (p=0·18; table 2).

Severities of solicited local and systemic adverse reactions reported within 28 days after the third dose were grade 1–2 in all vaccination cohorts in both trials. The most common reported reaction was injection-site pain (table 3; appendix 4 pp 22–28). Taking the 3 µg group as an example, the incidences of adverse reactions within 28 days after the third dose in primary three-dose regimens were five (9%) of 55 participants in cohort 1a-14d-2m and three (6%) of 54 participants in cohort 2a-28d-2m; not higher than the incidence of adverse reactions within 28 days after each previous dose (table 3; appendix 4 pp 22–23, 25–26). The overall incidence of any adverse reaction within 28 days after the booster dose (3 µg) was ten (18%) of 55 participants in cohort 1b-14d-8m, eight (15%) of 52 in cohort 2b-28d-8m, and five (6%) of 90 in cohort 3-28d-8m (table 3; appendix 4 p 24, 27–28).

Serious adverse events were reported in one (2%) of 60 participants in the 3 µg group and two (3%) of 60 participants in the 6 µg group in cohort 1a-14d-2m, in two (3%) of 60 participants in the 3 µg group and two (3%) of 60 in the 6 µg group in cohort 1b-14d-8m, and in no participant in the 30 µg group and one (2%) of 60 in the 6 µg group in each of cohorts 2a-28d-2m and 2b-28d-8m (appendix 4 pp 29–30). No participant in the placebo group reported a serious adverse event. From the beginning of immunisation to 28 days after dose 3 in cohort 3-28d-8m, ten (10%) of 100 participants in the 1·5 µg group, five (5%) of 101 in the 3 µg group, seven (7%) of 99 in the 6 µg group, and two (4%) of 49 in the placebo group had non-fatal serious adverse events (appendix 4 pp 30–31). No serious adverse event in either trial was considered by the investigators to be related to vaccination, and no prespecified trial-halting rules were met.

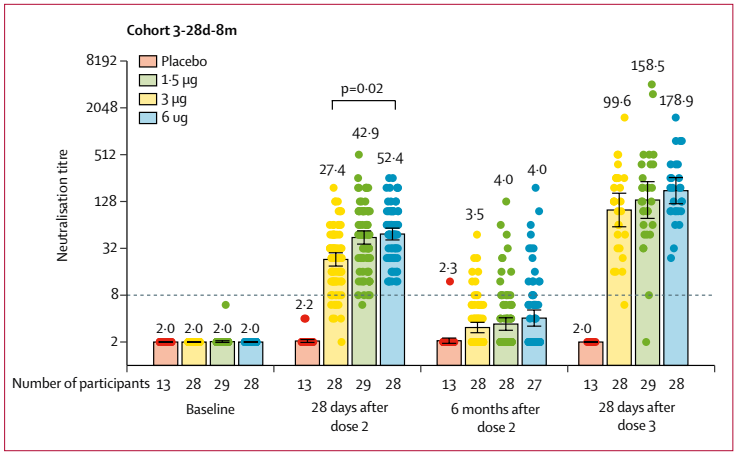


Figure 3: **Level of neutralising antibodies to infectious SARS-CoV-2 in adults aged 60 years and older**
Dots are reciprocal neutralising antibody titres for individuals in the per-protocol population. Numbers above the bars are GMTs, and the error bars indicate the 95% CI. The dotted horizontal line represents the seropositivity threshold. Titres lower than the limit of detection (1/4) are presented as half the limit of detection. Numbers above the short horizontal lines are p values of comparisons between 1.5 µg group, 3 µg group, and 6 µg group. Only the p values indicating significant difference are marked. GMT=geometric mean titre.

Discussion

Our study showed that the initial neutralising antibody response from two doses of CoronaVac declined to near or below the lower limit of seropositivity after 6 months. A third dose of CoronaVac (3 µg) given 8 months after the second dose led to a strong boost in immunity, with neutralising GMTs increasing to approximately 140 among adults aged 18–59 years and 159 among adults aged 60 years and older 14–28 days after the booster dose. These increases correspond to roughly three-fold to five-fold increases in neutralising antibody titres compared with titres 28 days after a second dose. Seropositivity 28 days after a third dose at 8 months was 98–100% regardless of age group. By contrast, a third dose given 2 months after the second dose induced much lower neutralising antibody titres. Reactogenicity of the third dose was indistinguishable from reactogenicity of the previous two doses, regardless of age group.

Decreases over time of vaccine-induced neutralising antibodies against ancestral SARS-CoV-2 have been observed with other COVID-19 vaccines, but at a much lower magnitude. For example, following vaccination with Moderna's mRNA-1273 vaccine, neutralising antibodies declined but remained detectable among all participants on days 90 and 180 after a second dose.[6,26] SARS-CoV-2 spike protein-specific memory B cells are detectable in most patients with COVID-19 and in people who are naive to SARS-CoV-2 after receiving two doses of COVID-19 vaccines.[27,28] This study is the first to show that the antibody response mediated by a third dose of CoronaVac given 2 months after the second dose rebounded only moderately and degraded to near the seropositive threshold after 6 months. This observation is probably because the interval between the two doses was short and the memory B cells were immature. However, a third dose of CoronaVac given 8 months after the second dose appears to effectively augment the potency, breadth, and likely duration of anamnestic responses against SARS-CoV-2.[29] Compared

	Cohort 1a-14d-2m			Cohort 1b-14d-8m			Cohort 2a-28d-2m			Cohort 2b-28d-8m			Cohort 3-28d-8m			
	3 µg (N=55)	6 µg (n=58)	Placebo (N=26)	3 µg (N=55)	6 µg (N=56)	Placebo (N=30)	3 µg (N=54)	6 µg (N=50)	Placebo (N=26)	3 µg (N=52)	6 µg (N=50)	Placebo (N=28)	1·5 µg (N=85)	3 µg (N=90)	6 µg (N=81)	Placebo (N=47)
Any adverse reaction																
Grade 1	5 (9%)	5 (9%)	0	10 (18%)	13 (23%)	3 (10%)	3 (6%)	1 (2%)	0	7 (13%)	10 (20%)	1 (4%)	3 (4%)	3 (3%)	3 (4%)	2 (4%)
Grade 2	1 (2%)	1 (2%)	0	1 (2%)	0	1 (3%)	0	0	0	1 (2%)	1 (2%)	1 (4%)	1 (1%)	2 (2%)	2 (2%)	1 (2%)
Systemic diseases and injection site adverse reactions																
Injection site pain	3 (5%)	5 (9%)	0	8 (1%)	9 (16%)	0	1 (2%)	1 (2%)	0	6 (12%)	7 (14%)	0	1 (1%)	2 (2%)	2 (2%)	1 (2%)
Injection site swelling	0	0	0	0	0	1 (3%)	0	0	0	1 (2%)	0	0	0	0	0	0
Injection site itch	0	0	0	0	1 (2%)	2 (7%)	0	0	0	1 (2%)	0	0	0	0	0	0
Injection site erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1%)	0	0
Fever	0	0	0	0	1 (2%)	0	0	0	0	1 (2%)	1 (2%)	1 (4%)	0	0	0	0
Fatigue	0	0	0	0	1 (2%)	0	1 (2%)	0	0	1 (2%)	2 (4%)	0	0	1 (1%)	0	1 (2%)
Respiratory, thoracic, and mediastinal disorders																
Cough	0	1 (2%)	0	0	2 (4%)	0	2 (4%)	0	0	0	0	0	1 (1%)	0	1 (1%)	1 (2%)
Runny nose	0	0	0	0	0	0	0	0	0	0	1(2%)	0	0	0	1(1%)	0
Oropharyngeal pain	0	0	0	0	1 (2%)	0	0	0	0	1 (2%)	0	0	0	0	0	0
Laryngeal stimulation	0	0	0	1 (2%)	0	0	0	0	0	0	0	0	0	0	0	0
Nervous system disorders																
Dizziness	0	0	0	0	1 (2%)	0	0	0	0	0	0	0	1 (1%)	1 (1%)	0	0
Headache	0	1 (2%)	0	1 (2%)	2 (4%)	1 (3%)	0	0	0	1 (2%)	1 (2%)	1 (4%)	0	0	1 (1%)	0 (0%)
Gastrointestinal disorders																
Diarrhoea	1 (2%)	0	0	0	0	0	1 (2%)	0	0	1 (2%)	0	0	0	0	0	0
Nausea	1 (2%)	1 (2%)	0	1 (2%)	0	0	1 (2%)	0	0	0	2 (4%)	0	1 (1%)	1 (1%)	0	0
Musculoskeletal and connective tissue disorders																
Muscle pain	1 (2%)	0	0	0	1 (2%)	0	0	0	0	0	0	0	0	0	0	0
Myalgia	0	0	0	0	0	0	0	0	0	0	1 (2%)	0	1 (1%)	0	0	0
Skin and subcutaneous tissue disorders																
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1%)	0
Eye disorders																
Periorbital oedema	0	0	0	0	1 (2%)	0	0	0	0	0	0	0	0	0	0	0

Data are n (%), representing the total number of participants who had adverse reactions (ie, adverse events related to vaccination).

Table 3: **Adverse reactions within 28 days after the third dose**

www.thelancet.com/infection **Published online December 7, 2021 https://doi.org/10.1016/S1473-3099(21)00681-2**

with the 3 µg formulation of CoronaVac, which is approved for use, the 1·5 µg formulation produced similar neutralising antibody titres by day 28 after the third dose for adults aged 60 years and older. Whether the 1·5 µg formulation could serve as a booster dose needs further study due to the small sample size in the analysis of this dose (28 participants).

Significant rebound in antibody concentration induced by homologous booster doses has been reported for other vaccines. Neutralisation titres against ancestral SARS-CoV-2 increased approximately four-fold after a homologous booster dose compared with titres following primary series with BNT162b2,[10] mRNA-1273,[11] and NVX-CoV2373,[12] with similarly long intervals (6–8 months) between the booster dose and primary vaccination. A nine-fold increase in spike protein-binding antibody was observed after a 6-month homologous booster dose of Ad26.COV2-S.[30]

Heterologous prime–boost regimens appear to induce higher levels of immune response than homologous booster doses. Vaccination with mRNA vaccines and adenovirus-vectored vaccines[31,32] or inactivated vaccines and adenovirus-vectored vaccines[33] have shown strong short-term immune responses and tolerable reactogenicity. Wanlapakorn and colleagues[34] found that CoronaVac and AZD1222 vaccine recipients had higher neutralising antibody activity against the original wild-type virus and the beta (B.1.351) variant of concern than did recipients of two doses of CoronaVac or AZD1222, suggesting that heterologous immunisation might be considered an alternative to homologous boosting for immunisation programmes. Long-term effectiveness of boosting remains unevaluated because of the newness of COVID-19 vaccine booster dosing.

SARS-CoV-2 continues to evolve and produce variants, among which the delta variant has become predominant.[9] Although we did not perform neutralisation testing in vitro against emerging variants of concern, high neutralising antibody titres against the ancestral strain are believed to be important for protection against novel circulating SARS-CoV-2 variants that potentially can lead to immune escape.[35] Several studies have reported in-vitro neutralisation titres against variants for CoronaVac, but results varied greatly. Vacharathit and colleagues, using a live-virus microneutralisation assay, identified 22-fold and 32-fold reductions in neutralising antibodies against the beta and delta variants, respectively, compared with ancestral SARS-CoV-2.[36] Wang and colleagues reported a three-fold reduction in neutralising antibody titres against the beta variant, using a pseudovirus neutralisation assay.[37] Another study reported 5·7-fold, 4·3-fold, and 3·7-fold reductions of neutralising antibody titres against beta, gamma (P.1), and delta variants, respectively.[29] Of note, it is difficult to directly compare these estimates because of the differences in study design and laboratory methods.[38] Determining the neutralisation ability of CoronaVac to emerging variants and evaluating the protection level in risk groups such as immunosuppressed individuals or elderly people are important research endeavours.

Decreased effectiveness of mRNA vaccines against SARS-CoV-2 infection with circulating variants has been seen in real-world studies in the USA, but effectiveness against hospitalisation was sustained.[39,40] Two doses of CoronaVac showed good effectiveness in a setting with co-circulating alpha and gamma variants in Chile: the vaccine was 66% effective against COVID-19 and nearly 90% effective against severe outcomes.[19] A test-negative case-control study done in Brazil showed that the adjusted vaccine effectiveness against hospital admission was above 55% in older adults during a time of extensive transmission of the gamma variant.[20] During local outbreaks caused by the delta variant in China, two studies with small sample sizes showed that inactivated vaccines were 70·2% effective against illness of moderate or worse severity[41] and could lower the risk of progressing to severe disease by 88%.[22] Protection against variants and persistence in protection with CoronaVac need to be continually evaluated in real-world studies.

Interim protection results from booster programmes in Israel showed that booster doses effectively reduced breakthrough infections, including breakthroughs of the delta variant.[14] Considering sustained protection of primary immunisation with COVID-19 vaccines against severe outcomes[42] and equity in vaccine deployment, WHO currently prioritises completion of primary immunisation over booster dose strategies to protect more people from COVID-19 due to global shortage of supply of COVID-19 vaccines,[43] although the US Centers for Disease Control and Prevention has issued booster recommendations for specific populations.[44]

During the trials, participants were masked to study group assignment and participants in placebo groups could be vaccinated immediately after completion of the phase 2 trial for adults aged 18–59 years and completion of follow-up for 28 days after the booster dose for adults aged 60 years and older. Since strict non-pharmaceutical interventions have been maintained to date across mainland China, the risk of infection was very low for participants in the placebo group. Maintenance of the placebo groups until the end of the trial was approved by Jiangsu Ethics Committee (JSJK2020-A021–02) and Hebei CDC Ethics Committee (IRB2020–006).

Our study has several limitations. First, establishment of SARS-CoV-2 spike protein-specific immune memory, in addition to inducing durable antibodies, might be important for a successful COVID-19 vaccine. For example, T-cell immunity elicited by inactivated vaccines might contribute to protection.[45,46] However, T-cell responses and neutralisation tests in vitro against emerging variants were not assessed in our study, and these need to be further explored. Second, we report the results of interim analyses, and long-term follow-up is ongoing to identify a satisfactory duration of immunity

induced by the booster dose and to assess longer-term safety. Third, a population at greatest risk of immunosenescence (ie, adults aged 80 years and older) was not evaluated in this study. Larger, multicentre studies will be needed to assess primary outcomes among subpopulations for whom our study had relatively small proportions. Fourth, although neutralising antibodies are related to protection, actual protection from infection with current and emerging variants will need to be monitored with real-world observational studies. Further research to identify correlates of protection and to determine whether different vaccines have different correlates is important.

In conclusion, our study found that a two-dose schedule of CoronaVac generated good immune memory. Although neutralising antibody titres decreased to near or below the lower limit of seropositivity 6 months after the second dose, a third dose given 8 months after the second dose was highly effective at recalling a SARS-CoV-2-specific immune response, leading to a significant rebound in antibody levels. Our study indicates that a homologous booster dose might provide longer-lasting immunity and higher levels of protection than a two-dose schedule, but additional study is needed to monitor neutralisation ability and effectiveness against variants.

Contributors

GZ, QW, HP, ML, JY, YZ, FZ, HY, and WY designed the study and contributed to data collection, data analysis, data interpretation, and writing of the manuscript. GZ, QW, HP, and ML verified the data. ZW, KC, LeW, and BH collected data and revised the manuscript. DJ and LiW did the laboratory assays and revised the manuscript. XD, WZ, and WL analysed the data and revised the manuscript. All authors had full access to all of the data (including statistical reports and tables) in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors had final responsibility for the decision to submit the manuscript for publication.

Declaration of interests

HY received research funding from Sanofi Pasteur, GlaxoSmithKline, Yichang HEC Changjiang Pharmaceutical Company, and Shanghai Roche Pharmaceutical Company; none of this research funding is related to development of COVID-19 vaccines. GZ, LeW and WY are employees of Sinovac Biotech and LiW and DJ are employees of Sinovac Life Sciences. All other authors declare no competing interests.

Data sharing

The individual participant-level data that underlie the results reported in this Article (text, tables, figures, and appendices) will be shared after de-identification. This clinical trial is ongoing, and all the individual participant data cannot be available until the immune persistence evaluation is done. The data will be available immediately after publication and finalisation of the completed clinical study report for at least 1 year. Supporting clinical documents, including the study protocol and statistical analysis plan, and the informed consent form will be available immediately following the publication of this Article for at least 1 year. Information on how to access supporting clinical documents is available online for adults aged 18–59 years at http://www.jshealth.com/ and for adults aged 60 years and older at http://www.hebeicdc.cn/kygz/22506.jhtml. Researchers who provide a scientifically sound proposal will be allowed access to the de-identified individual participant data. Proposals should be sent to the corresponding authors. These proposals will be reviewed and approved by the sponsor, investigators, and collaborators on the basis of scientific merit. To gain access, data requestors will need to sign a data access agreement.

Acknowledgments

The study was supported by grants from National Key Research and Development Program (2020YFC0849600), Beijing Science and Technology Program (Z201100005420023), and Key Program of the National Natural Science Foundation of China (82130093). We thank Dr Lance Rodewald from Chinese Center for Disease Control and Prevention for his comments and English language editing.

References

1 UNICEF. COVID-19 vaccine market dashboard. https://www.unicef.org/supply/covid-19-vaccine-market-dashboard (accessed Oct 1, 2021).
2 Our World in Data. Statistics and research, coronavirus (COVID-19) vaccinations. https://ourworldindata.org/covid-vaccinations (accessed Oct 3, 2021).
3 Favresse J, Bayart JL, Mullier F, et al. Antibody titres decline 3-month post-vaccination with BNT162b2. *Emerg Microbes Infect* 2021; **10:** 1495–98.
4 Nanduri S, Pilishvili T, Derado G, et al. Effectiveness of Pfizer-BioNTech and Moderna vaccines in preventing SARS-CoV-2 infection among nursing home residents before and during widespread circulation of the SARS-CoV-2 B.1.617.2 (delta) variant—national healthcare safety network, March 1–August 1, 2021. *MMWR Morb Mortal Wkly Rep* 2021; **70:** 1163–66.
5 Tartof SY, Slezak JM, Fischer H, et al. Effectiveness of mRNA BNT162b2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA: a retrospective cohort study. *Lancet* 2021; **398:** 1407–16.
6 Doria-Rose N, Suthar MS, Makowski M, et al. Antibody persistence through 6 months after the second dose of mRNA-1273 vaccine for COVID-19. *N Engl J Med* 2021; **384:** 2259–61.
7 Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of COVID-19 vaccines against the B.1.617.2 (delta) variant. *N Engl J Med* 2021; **385:** 585–94.
8 Flaxman A, Marchevsky NG, Jenkin D, et al. Reactogenicity and immunogenicity after a late second dose or a third dose of ChAdOx1 nCoV-19 in the UK: a substudy of two randomised controlled trials (COV001 and COV002). *Lancet* 2021; **398:** 981–90.
9 Chen Z, Azman AS, Chen X, et al. Landscape of SARS-CoV-2 genomic surveillance, public availability extent of genomic data, and epidemic shaped by variants: a global descriptive study. *medRxiv* 2021; published online Sept 8. https://www.medrxiv.org/content/10.1101/2021.09.06.21263152v1 (preprint).
10 Falsey AR, Frenck RW Jr, Walsh EE, et al. SARS-CoV-2 neutralization with BNT162b2 vaccine dose 3. *N Engl J Med* 2021; **385:** 1627–29.
11 Moderna. Business updates, second quarter 2021 financial results. Aug 5, 2021. https://investors.modernatx.com/static-files/c43de312-8273-4394-9a58-a7fc7d5ed098 (accessed Aug 20, 2021).
12 NOVAVAX. Second quarter 2021 financial results and operational highlights. Aug 5, 2021. https://filecache.investorroom.com/mr5ir_novavax/115/Q2-2021-Earnings-Slides.pdf (accessed Aug 20, 2021).
13 Israel National News. Booster program expands: Israel expands third vaccine dose to all adults over 30. Aug 24, 2021. https://www.israelnationalnews.com/News/News.aspx/312324 (accessed Sept 2, 2021).
14 Bar-On YM, Goldberg Y, Mandel M, et al. Protection of BNT162b2 vaccine booster against COVID-19 in Israel. *N Engl J Med* 2021; **385:** 1393–400.
15 China Food and Drug Administration. Conditional use approval for CoronaVac. https://www.nmpa.gov.cn/yaopin/ypjgdt/20210206154636109.html (accessed July 24, 2021).
16 WHO. Regulation and prequalification. https://www.who.int/teams/regulation-prequalification/eul/covid-19 (accessed Aug 20, 2021).
17 Gavi, the Vaccine Alliance. COVAX global supply forecast. Sept 8, 2021. https://www.gavi.org/sites/default/files/covid/covax/COVAX-Supply-Forecast.pdf (accessed July 21, 2021).
18 China Food and Drug Administration. COVID-19 vaccination status (as of October 3). http://www.gov.cn/xinwen/2021-10/04/content_5640964.htm (accessed Oct 3, 2021).
19 Jara A, Undurraga EA, González C, et al. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. *N Engl J Med* 2021; **385:** 875–84.

20 Ranzani OT, Hitchings MDT, Dorion M, et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of COVID-19 in Brazil: test negative case-control study. *BMJ* 2021; **374:** n2015.

21 Kang M, Yi Y, Li Y, et al. Effectiveness of inactivated COVID-19 vaccines against COVID-19 pneumonia and severe illness caused by the B.1.617.2 (delta) variant: evidence from an outbreak in Guangdong, China. *SSRN* 2021; published online Aug 5. https://ssrn.com/abstract=3895639 (preprint).

22 Hu Z, Tao B, Li Z, et al. Effectiveness of inactive COVID-19 vaccines against severe illness in B.1.617.2 (delta) variant-infected patients in Jiangsu, China. *medRxiv* 2021; published online Sept 5. https://www.medrxiv.org/content/10.1101/2021.09.02.21263010v1.full (preprint).

23 Zhang Y, Zeng G, Pan H, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21:** 181–92.

24 Wu Z, Hu Y, Xu M, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21:** 803–12.

25 WHO. Polio laboratory manual, 4th ed. 2004. https://apps.who.int/iris/handle/10665/68762 (accessed Aug 17, 2021).

26 Widge AT, Rouphael NG, Jackson LA, et al. Durability of responses after SARS-CoV-2 mRNA-1273 vaccination. *N Engl J Med* 2021; **384:** 80–82.

27 Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 2021; **371:** eabf4063.

28 Goel RR, Apostolidis SA, Painter MM, et al. Distinct antibody and memory B cell responses in SARS-CoV-2 naive and recovered individuals following mRNA vaccination. *Sci Immunol* 2021; **6:** eabi6950.

29 Wang K, Cao Y, Zhou Y, et al. A third dose of inactivated vaccine augments the potency, breadth, and duration of anamnestic responses against SARS-CoV-2. *medRxiv* 2021; published online Sept 5. https://www.medrxiv.org/content/10.1101/2021.09.02.21261735v1 (preprint).

30 Sadoff J, Le Gars M, Cardenas V, et al. Durability of antibody responses elicited by a single dose of Ad26.COV2.S and substantial increase following late boosting. *medRxiv* 2021; published online Aug 26. https://www.medrxiv.org/content/10.1101/2021.08.25.21262569v1 (preprint).

31 Barros-Martins J, Hammerschmidt SI, Cossmann A, et al. Immune responses against SARS-CoV-2 variants after heterologous and homologous ChAdOx1 nCoV-19/BNT162b2 vaccination. *Nat Med* 2021; **27:** 1525–29.

32 Borobia AM, Carcas AJ, Pérez-Olmeda M, et al. Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (CombiVacS): a multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet* 2021; **398:** 121–30.

33 Li J, Hou L, Guo X, et al. Heterologous prime–boost immunization with CoronaVac and Convidecia. *medRxiv* 2021; published online Sept 6. https://doi.org/10.1101/2021.09.03.21263062 (preprint).

34 Wanlapakorn N, Suntronwong N, Phowatthanasathian H, et al. Immunogenicity of heterologous prime/booster-inactivated and adenoviral-vectored COVID-19 vaccine: real-world data. *Research Square* 2021; published online Sept 10. https://www.researchsquare.com/article/rs-785693/v1 (preprint).

35 Choi A, Koch M, Wu K, et al. Safety and immunogenicity of SARS-CoV-2 variant mRNA vaccine boosters in healthy adults: an interim analysis. *Nat Med* 2021; published online Sept 15. https://doi.org/10.1038/s41591-021-01527-y.

36 Vacharathit V, Aiewsakun P, Manopwisedjaroen S, et al. CoronaVac induces lower neutralising activity against variants of concern than natural infection. *Lancet Infect Dis* 2021; **21:** 1352–54.

37 Wang G-L, Wang Z-Y, Duan L-J, et al. Susceptibility of circulating SARS-CoV-2 variants to neutralization. *N Engl J Med* 2021; **384:** 2354–56.

38 Chen X, Chen Z, Azman AS, et al. Neutralizing antibodies against SARS-CoV-2 variants induced by natural infection or vaccination: a systematic review and pooled meta-analysis. *Clin Infect Dis* 2021; published online July 24. https://doi.org/10.1093/cid/ciab646.

39 Tenforde MW, Self WH, Naioti EA, et al. Sustained effectiveness of Pfizer-BioNTech and Moderna vaccines against COVID-19 associated hospitalizations among adults—United States, March–July 2021. *MMWR Morb Mortal Wkly Rep* 2021; **70:** 1156–62.

40 Eli S, David H, Vajeera D, et al. New COVID-19 cases and hospitalizations among adults, by vaccination status—New York, May 3–July 25, 2021. *MMWR Morb Mortal Wkly Rep* 2021; **70:** 1150–55.

41 Li XN, Huang Y, Wang W, et al. Efficacy of inactivated SARS-CoV-2 vaccines against the delta variant infection in Guangzhou: a test-negative case-control real-world study. *Emerg Microbes Infect* 2021; **10:** 1751–59.

42 Krause PR, Fleming TR, Peto R, et al. Considerations in boosting COVID-19 vaccine immune responses. *Lancet* 2021; **398:** 1377–80.

43 WHO. Interim statement on COVID-19 vaccine booster doses. Aug 10, 2021. https://www.who.int/news/item/10-08-2021-interim-statement-on-covid-19-vaccine-booster-doses (accessed Aug 20, 2021).

44 Centres for Disease Control and Prevention. CDC statement on ACIP booster recommendations. Sept 24, 2021. https://www.cdc.gov/media/releases/2021/p0924-booster-recommendations-.html (accessed Oct 4, 2021).

45 Quast I, Tarlinton D. B cell memory: understanding COVID-19. *Immunity* 2021; **54:** 205–10.

46 Deng Y, Li Y, Yang R, Tan W. SARS-CoV-2-specific T cell immunity to structural proteins in inactivated COVID-19 vaccine recipients. *Cell Mol Immunol* 2021; **18:** 2040–41.

7.7. Dose de reforço da CoronaVac aumenta mais de 12 vezes o nível de anticorpos de quem tomou duas doses da vacina

Pesquisadores chilenos, americanos e chineses constataram que a dose de reforço da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, aumenta em mais de 12 vezes o nível de anticorpos de quem tomou as duas doses do imunizante há pelo menos cinco meses. Os resultados do estudo "A booster dose of an inactivated vaccine increases neutralizing antibodies and T cell responses against SARS-CoV-2" foram publicados na plataforma de preprints medRxiv.

"Após a dose de reforço, a capacidade de neutralização dos anticorpos aumentou ainda mais do que a relatada duas semanas após a segunda dose. Observamos que, quatro semanas após a dose de reforço, a capacidade neutralizante aumentou mais de 12 vezes em comparação com a resposta cinco meses após a segunda dose, e aumentou mais de duas vezes em comparação com os níveis registrados duas semanas após a segunda dose", afirmam os pesquisadores, do Instituto Milênio de Imunologia e Imunoterapia, da Pontifícia Universidade Católica do Chile; do Instituto de Imunologia La Jolla, da Universidade da Califórnia em San Diego, nos Estados Unidos; e da Sinovac.

O estudo foi realizado com 129 voluntários que receberam a primeira dose da CoronaVac de janeiro a março de 2021, e a segunda com um intervalo de 28 dias. Decorridos cinco meses, os voluntários tomaram a dose de reforço. A capacidade de neutralização de anticorpos foi avaliada em 77 voluntários.

Em adultos entre 18 e 59 anos de idade, a capacidade de neutralização dos anticorpos circulantes atingiu seu máximo quatro semanas após a dose de reforço, aumentando mais de 18 vezes em comparação com os níveis registrados cinco meses após a segunda dose, e mais de quatro vezes em comparação com os níveis registrados duas semanas após a segunda dose. A soropositividade nesse grupo chegou a 100% quatro semanas após a segunda dose.

Em um esquema normal de imunização de duas doses com intervalo de 28 dias, o pico na capacidade de neutralização dos anticorpos é atingido duas semanas após a segunda dose. Entre maiores de 60 anos, que correspondiam a 53,2% dos voluntários, os pesquisadores observaram que após a dose de reforço houve um aumento de mais de nove vezes na capacidade neutralizante em relação à resposta observada cinco meses após a segunda dose.

Publicado em: 17/11/2021

A booster dose of an inactivated vaccine increases neutralizing antibodies and T cell responses against SARS-CoV-2.

Bárbara M Schultz[1,#], Felipe Melo-González[1,#], Luisa F Duarte[1,#], Nicolás MS Gálvez[1,#], Gaspar A Pacheco[1,#], Jorge A Soto[1,#], Roslye V Berríos-Rojas[1,2], Liliana A González[1,2], Daniela Moreno-Tapia[1,2], Daniela Rivera-Pérez[1,2], Guillermo Hoppe-Elsholz[1,2], Carolina Iturriaga[3], Mariana Ríos[1,2], Omar P Vallejos[1,2], Marcela Urzua[3], Yaneisi Vázquez[1,2], María S Navarrete[4], Álvaro Rojas[4], Daniela Weiskopf[5], Alessandro Sette[5,6], Gang Zeng[7], Weining Meng[7], CoronaVac03CL Study Group, José V González-Aramundiz[8], Pablo A González[1,2], Katia Abarca[1,3], Alexis M Kalergis*[1,2,9], Susan M Bueno*[1,2]

Affiliations:

[1]Millennium Institute on Immunology and Immunotherapy, Santiago, Chile. [2]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile. [3]Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, División de Pediatría, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile. [4]Departamento de Enfermedades Infecciosas del Adulto, División de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile. [5]Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology (LJI), La Jolla, CA 92037, USA. [6]Department of Medicine, Division of Infectious Diseases and Global Public Health, University of California, San Diego (UCSD), La Jolla, CA 92037, USA. [7]Sinovac Biotech, Beijing, China.

[8]Departamento de Farmacia, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile. [9]Departamento de Endocrinología, Facultad de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.

[#] These authors contributed equally to this work.

*Corresponding authors.

Keywords:

CoronaVac; Phase 3 clinical trial; SARS-CoV-2; COVID-19; Vaccines, Booster, Third dose.

Abstract

Numerous vaccines have been generated to decrease the morbidity and mortality of COVID-19. CoronaVac® is an inactivated SARS-CoV-2 vaccine approved by the World Health Organization (WHO) to prevent COVID-19 that has safety and immunogenicity profiles described in different clinical trials. We previously reported an increase in levels of neutralizing antibodies two- and four-weeks after administering two doses of CoronaVac® in a two-week interval (0-14 day) vaccination schedule, as compared to pre-immune sera in adults in the Chilean population that are participating in a phase 3 clinical trial. Here we report the levels of antibodies directed against the Receptor Binding Domain of the SARS-CoV-2 spike protein comparing their neutralizing capacities and the cellular response at five months after the second dose and four weeks after a booster (third) dose in volunteers immunized with two doses of CoronaVac® in a four-week interval (0-28 day) vaccination schedule. We observed a decrease in the levels of anti-SARS-CoV-2 antibodies with neutralizing capacities five months after the second dose (GMU 39.0 95% confidence interval (CI)□(32.4-47.0), which increased up to 12 times at four weeks after the booster dose (GMU 499.4, 95% CI=370.6-673.0). Equivalent results were observed in adults aged 18-59 years old and individuals ≥60 years old. In the case of cellular response, we observed that activation of specific $CD4^+$ T cell increases in time and reaches its maximum at four weeks after the booster dose in both groups. Our results support the notion that a booster dose of the SARS-CoV-2 inactivated vaccine increases the levels of neutralizing antibodies and the specific cellular response in adults of both groups, which is likely to boost the protective capacity of these vaccines against COVID-19.

Introduction

The ongoing pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has promoted the rapid development of safe, immunogenic, and effective vaccines against SARS-CoV-2 to be used by the general population, which have successfully reduced the transmission of the disease burden. CoronaVac® is an inactivated SARS-CoV-2 vaccine developed by Sinovac Life Sciences Co., Ltd. (Beijing, China) and is among the current vaccines approved by the WHO to combat COVID-19 [1,2]. Phase 1 and 2 clinical trials in China demonstrated that this vaccine induces cellular and humoral response upon immunization [3–5]. Furthermore, an ongoing phase 3 clinical trial in Chile has described that two- and four-weeks after the second dose of CoronaVac® there is an increase in the levels of IgG and neutralizing antibodies in adults aged 18-59 years old and ≥ 60 years old [5][6]. In addition, the vaccination promotes the activation of the cellular immune response against SARS-CoV-2 antigens in a 0-14 immunization schedule [5], being an effective vaccine to prevent COVID-19 [7,8]. In Chile, 91.5% of the target population has received the first vaccine dose, and 88.7% were fully vaccinated in October 2021 in a 0-28 vaccination schedule [9]. Although neutralizing antibody titers present in the serum of vaccinated people are thought to be highly predictive of immune protection [10], these titers decrease in time [6,11,12]. Besides this, vaccine-induce antibodies have lower levels of neutralization against highly transmissible variants of the virus as compared to the original vaccine strain, potentially decreasing the effectiveness of these vaccines as new variants emerge [13,14]. For these reasons, the use of booster doses was

approved in adults in August 2021 in Chile, in high-risk populations and subjects with more than five months after the second dose applied in a 0-28-day vaccination schedule [15]. Notably, a previous study performed in adults between 18-59 years old demonstrates that a booster dose of CoronaVac®, applied after six months to individuals previously receiving two doses of this vaccine, increases the levels of antibodies 3-5-fold as compared to those levels observed four weeks after the second dose [12]. Here, we further extend these results by reporting the levels of neutralizing antibodies and specific T cells against SARS-CoV-2 in adults ≥18 years old who participated in phase 3 clinical trial carried out in Chile, who were vaccinated in a 0-28-day vaccination schedule with a booster (third) dose five months after the second dose.

Materials and methods

Patients and sample collection

Blood samples were obtained from volunteers recruited in the clinical trial CoronaVac03CL (clinicaltrials.gov #NCT04651790) carried out in Chile starting January 2021. The Institutional Scientific Ethical Committee of Health Sciences reviewed and approved the study protocol at the Pontificia Universidad Católica de Chile (#200708006). Trial execution was approved by the Chilean Public Health Institute (#24204/20) and was conducted according to the current Tripartite Guidelines for Good Clinical Practices, the Declaration of Helsinki [16], and local regulations. Informed consent was obtained from all volunteers upon enrollment. Volunteers receive two doses of CoronaVac® (3 µg or 600SU of inactivated SARS-

CoV-2 inactivated along with alum adjuvant) in a four-week interval (0–28-day immunization schedule) and then a booster dose five months after the second dose. A complete inclusion and exclusion criteria list has been reported. On November 11st 2021, one hundred and eighty-six volunteers in the immunogenicity branch received the booster dose, and the antibodies against RBD with neutralizing capacities were quantified in 77 volunteers who had completed all their previous visits in one of the centers of the study (**Figure 1A**). Blood samples were obtained from all the volunteers before administration of the first dose (pre-immune), two weeks after the second dose, four weeks after the second dose, twenty weeks (or five months) after the 2nd dose, and four weeks after the booster (third) dose (**Figure 1B**).

Procedures

To assess the presence of antibodies against RBD with neutralizing capacities, blood samples from 77 volunteers that had completed all their study visits, including one month after the booster dose of CoronaVac®, were measured. The neutralizing capacities of circulating antibodies were evaluated by a surrogate virus neutralization test (sVNT) (Genscript Cat#L00847-A). Samples were serially two-fold diluted starting at a 4-fold until reaching a 512-fold dilution. Assays were performed according to the instructions of the manufacturer and as reported previously [5]. Neutralizing antibody titers were determined as the last fold dilution with a cut-off over 30% of inhibition. Samples with a percentage of inhibition ≤30 at lowest dilution (1:4) were assigned as seronegative with a titer of 2. A sample was considered seropositive when its titer is higher than the pre-immune titer. The

percentage of inhibition was determined as: 100 * [OD450nm value of negative control - OD450nm value of sample] / [OD450nm of negative control]. A standard curve was used to plot the neutralization response in the samples as international units (IU) by using the WHO International Standard for SARS-CoV-2 antibody (NIBSC code 20/136), which was prepared according to the manufacturer's instructions [17]. Data were analyzed using a sigmoidal curve model with log concentration transformed, and the final concentration for each sample was the average of the product of the interpolated IU from the standard curve and the sample dilution factor required to achieve the OD450 value that falls within the linear range. Samples with undetermined concentration at the lowest dilution tested (1:4) were assigned the lower limit of quantification (16.4 IU). The Geometric Mean Units (GMU) or titers (GMT) were represented in the **Figure 2** and **Supplementary Figure 1,** respectively**,** and **Table 1** for comparisons among the visits.

ELISPOT and flow cytometry assays were performed to evaluate the cellular immune response, stimulating PBMCs with four Mega Pools (MPs) of peptides derived from the proteome of SARS-CoV-2 [18]: peptides from the S protein of SARS-CoV-2 (MP-S), the remaining proteins of the viral particle (excluding S protein peptides) (MP-R), and of peptides from the whole proteome of SARS-CoV-2 (MP-CD8-A and MP-CD8-B) [18]. Positives and negative controls were held for each assay. The number of Spot Forming cells (SFC) for IFN-γ and IL-4 were determined by ELISPOT, and the expression of Activation-Induced Markers (AIM+) by T cells was evaluated by flow cytometry. Assays were performed according to the instructions of the manufacturer and as reported previously [5]. Further details

on the ELISPOT assay, antibodies used for flow cytometry, and the respective protocols can be found in the **Supplementary Table 1**.

Statistical analyses

Statistical differences for the immunogenicity results considered one-way ANOVAs mixed-effects analysis for comparisons between the booster dose and the other visits performed on the logarithms of the data. The significance level was set at 0.05 for all the analyses. All data were analyzed with GraphPad Prism 9.0.1.

Results

One hundred and twenty-nine volunteers from the immunogenicity branch, who received the booster dose of the CoronaVac®, were included in this study. The first dose of the vaccine was inoculated from January - March of 2021, and the second dose was inoculated 28 days after the first one. Of them, we evaluated the neutralization capacity of circulating antibodies in 77 volunteers at five different time points indicated previously by sVNT and 33 of the same volunteers by ELISPOT and flow cytometry (**Figure 1B**).

In a normal 0-28-day schedule, the peak in the neutralizing capacity of the antibodies is reached at two weeks after the second dose (GMT 25.8, 95% CI=19.5-34.2) (**Supp. Figure 1**), decreasing at four weeks after the second dose (GMT 16.6, 95% CI=13.1-21.0). However, this neutralizing capacity present an important decreased five months after the second dose (GMT 3.5, 95% CI=3.0-4.1), which is in line with previous reports where the immunity against SARS-CoV-2 wanes six months after infection or vaccination [19,20]. As expected, after the

booster dose, the neutralizing capacity of the antibodies increased even more than the one reported two weeks after the second dose. When we expressed the neutralizing capacity in arbitrary units of WHO (**Figure 2**) we observed that four weeks after the booster dose the neutralizing capacity increased more than 12-fold (GMU 499.4, 95% CI=370.6-673.0), as compared to the response at five months after the second dose (GMU 39.0 ± 32.4-47.0) and more than 2-fold as compared to the two weeks after the second dose (GMU 168.0 ± 126.8-222.5) (**Figure 2A**).

In adults between 18-59 years old, the neutralizing capacity of circulating antibodies reach its maximum four weeks after the booster dose (GMU 918.8 ± 623.4-1354) increasing more than 18-fold as compared to five months after the second dose (48.9 ± 37.6-63.5) and more than 4-fold as compared with two weeks after the second dose (GMU 220.2 ± 150.7-321.7) (**Figure 2B**). Seropositivity in this group reach 100% four weeks after the second dose (**Table 1**). 53.2% of the total volunteer analyzed here were adults ≥60 years. As seen in **Figure 2C**, the neutralizing capacity of circulating antibodies in this population also reached its peak at two weeks after the second dose (GMU 134.1 ± 89.2-201.6), decreasing at four weeks after the second dose (GMU 104.1 ± 71.8-151.0), and reaching its minimum at five months after the second dose (GMU 32.4 ± 25.1-41.8). In this group, we also observed an increase of more than 9-fold (GMU 300.5 ± 203.5-443.6) in the neutralizing capacity as compared to the response observed five months after the second dose (GMU 32.4). The seropositivity rate reached 49.4% in the total vaccine group and 35.7% in adults ≥60 years at five months after the second dose, which increased to 97.4% and 95.2%, respectively, four weeks after

the booster dose (**Table 1**). The seropositivity rate achieved at four weeks after the booster dose was the highest when compared with the other visits in the study in the total vaccinated group and in both groups analyzed.

Here we also report cellular responses following the booster dose of CoronaVac®, which is the first report of T cell responses in subjects vaccinated with a third dose of CoronaVac® to our knowledge. We did observe a significantly further increase in $CD4^+$ T cell activation in both age groups following the third booster dose by flow cytometry (**Figure 3**) but we did not see a further increase in IFN-γ production upon stimulation with S and R MPs by ELISPOT at that time point (**Supp. Figure 2**). In addition, $CD4^+$ T cell activation was still significantly increased 5 months after the 2^{nd} dose in both age groups, suggesting that the 0-28 schedule can stimulate $CD4^+$ cell responses over time. Moreover, we observed a significant increase in $CD8^+$ AIM^+ T cells following the third dose as compared to the time point 2 weeks following the second booster but not as compared to the pre-immune, whereas we did not observe a significant increase in IFN-γ upon stimulation with CD8 MPs at any time point, suggesting that CoronaVac promotes a reduced $CD8^+$ T cell responses, even after a third dose. Thus, although humoral responses decrease over time following vaccination with CoronaVac®, $CD4^+$ T cell responses stay significantly increased as compared to the pre-immune and the booster dose increases at least their activation.

Discussion

Although there was an adequate neutralization titer of anti-SARS-CoV-2 antibodies after two doses of CoronaVac® in the 0-28 schedule, with a 65.9% of effectiveness of preventing COVID-19 [8], the GMT waned in time, which was observed five months after the second dose. Due to this decrease in neutralizing capacity, a booster dose of CoronaVac® was evaluated in a clinical study in China, showing promising results in humoral immune responses [12]. The evaluation of the neutralization capacities reported here shows that after the booster dose, the neutralizing titers and seroconversion rates increase in the whole group even higher than two weeks after the second dose where was observed the peak in neutralization. As the neutralizing antibody titers correlate with protection against SARS-CoV-2 infection [10], these results likely imply a better outcome and protection against illness, as reported in previous studies performed in Israel that showed a decrease in the transmission and the severe disease by COVID-19 twelve or more days after booster inoculation [21]. Another study, performed with a booster dose of CoronaVac®, showed that an additional dose induced a good neutralization against SARS-CoV-2 WT strain and against variants four weeks after the booster dose, generating a long-lasting humoral response that was due to an enhancement of the memory immune response generated by B cells [22].

Adults ≥60 years old produced lower levels of antibodies with neutralizing capacities than the whole group during this study, which was also described in Bueno et al. [5]. This result is in line with previous data reported for a population vaccinated in Chile [6], a study among hospital workers who received two doses of CoronaVac® [23], and with the mRNA-1273 vaccine [24]. In this sense, our results are equivalent to those described in a phase 1/2 of the clinical trial with

CoronaVac®, showing that the neutralizing antibody titers in this group decrease at five months after the second dose and that a booster dose is required 6-8 months after the first vaccination to rapidly increased and steadily the neutralizing antibody titers [25].

In the case of cellular response, other studies have shown that Pfizer BNT162b2 and mRNA-1273 induce durable $CD4^+$ T cell activation and cytokine production up to six months following vaccination but it remains to be elucidated whether $CD4^+$ AIM^+ T cells and cytokine production further increase following a booster dose of these vaccines [26,27]. In contrast to these vaccines, CoronaVac® delivers not only the Spike protein but other viral antigens, which may explain why vaccinated individuals still display $CD4^+$ AIM^+ T cells five months after the second dose, without even a third dose.

Our report shows that the booster dose with CoronaVac® in a 0-28 schedule induces a higher production of antibodies with neutralizing capacities, which are higher than the levels observed with 2- and 4-weeks after the first doses, generating an increased humoral response even in adults ≥60 years old. Besides this, our results suggest that a third dose of CoronaVac® supports $CD4^+$ T cell activation, which may confer either protection or enhanced immune responses against the virus and prevent severe disease following SARS-CoV-2 exposure.

Limitations

This study presents some limitations, such as the reduced sample size for the assays. The assessment of total antibody response against Spike proteins and other SARS-CoV-2 proteins would also add additional information about the

humoral immune response against SARS-CoV-2 after the booster dose. Due to the limit of quantification of the technique, samples with undetermined concentration at the lowest dilution tested (1:4) were assigned the lower limit of quantification (16.4 IU) and other neutralization assays, such as conventional neutralization test, would confirm our results with the surrogate neutralization test used in this study.

Funding

This work was supported by: The Ministry of Health, Government of Chile supported the funding of the CoronaVac03CL Study; The Confederation of Production and Commerce (CPC), Chile, supported the funding of the CoronaVac03CL Study; the NIH NIAID under Contract No. 75N93021C00016 supports AS and Contract No. 75N9301900065 supports AS, DW. The Millennium Institute on Immunology and Immunotherapy, ANID - Millennium Science Initiative Program ICN09_016 (former P09/016-F) supports SMB, KA, PAG and AMK; The Innovation Fund for Competitiveness FIC-R 2017 (BIP Code: 30488811-0) supports SMB, PAG and AMK; SINOVAC contributed to this study with the investigational vaccine and experimental reagents.

Competing interests

ZG and MW are SINOVAC employees and contributed to the conceptualization of the study (clinical protocol and eCRF design) and did not participate in the analysis or interpretation of the data presented in the manuscript. All other authors declare no conflict of interest. A.S. is a consultant for Gritstone, Flow Pharma, Arcturus,

Immunoscape, CellCarta, OxfordImmunotech and Avalia. Jolla Institute for Immunology (LJI) has filed for patent protection for various aspects of T cell epitope and vaccine design work. All other authors declare no conflict of interest. The authors declare this study received the investigational product (placebo and vaccines) from the company SINOVAC Biotech. SINOVAC employees contributed to the conceptualization of the study (clinical protocol and eCRF design) but did not participate in either the analysis or interpretation of the data shown in this manuscript.

Acknowledgments

We would like to thank the support of the Ministry of Health, Government of Chile; Ministry of Science, Technology, Knowledge, and Innovation, Government of Chile; and The Ministry of Foreign Affairs, Government of Chile, and the Chilean Public Health Institute (ISP). We also would like to thank Rami Scharf, Jessica White, Jorge Flores and Miren Iturriza-Gomara from PATH for their support on experimental design and discussion. We also thank the Vice Presidency of Research (VRI), the Direction of Technology Transfer and Development (DTD), the Legal Affairs Department (DAJ) of the Pontificia Universidad Católica de Chile. We are also grateful to the Administrative Directions of the School of Biological Sciences and the School of Medicine of the Pontificia Universidad Católica de Chile for their administrative support. Members of the CoronaVac03CL Study Group are listed in the Supplementary Appendix (SA). We would also like to thank the members of the independent data safety monitoring committee (members in the SA) for their oversight, and the subjects enrolled in the study for their

participation and commitment to this trial. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. Contract No. 75N9301900065 to A.S, D.W.

References

[1] Mallapaty S. WHO approval of Chinese CoronaVac COVID vaccine will be crucial to curbing pandemic [Internet]. Nature. Nature; 2021 [cited 2021 Oct 6]. p. 161–162. Available from: https://pubmed.ncbi.nlm.nih.gov/34089030/.

[2] Q G, L B, H M, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science [Internet]. 2020 [cited 2021 Oct 6];369:77–81. Available from: https://pubmed.ncbi.nlm.nih.gov/32376603/.

[3] Z W, Y H, M X, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis [Internet]. 2021 [cited 2021 Oct 6];21:803–812. Available from: https://pubmed.ncbi.nlm.nih.gov/33548194/.

[4] Y Z, G Z, H P, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis [Internet]. 2021 [cited 2021 Oct 6];21:181–192. Available from: https://pubmed.ncbi.nlm.nih.gov/33217362/.

[5] SM B, K A, PA G, et al. Safety and Immunogenicity of an Inactivated SARS-CoV-2 Vaccine in a Subgroup of Healthy Adults in Chile. Clin Infect Dis [Internet]. 2021 [cited 2021 Oct 9]; Available from: https://pubmed.ncbi.nlm.nih.gov/34537835/.

[6] Sauré D, O'Ryan M, Torres JP, et al. Dynamic IgG seropositivity after rollout of CoronaVac and BNT162b2 COVID-19 vaccines in Chile: a sentinel surveillance study. Lancet Infect Dis [Internet]. 2021 [cited 2021 Oct 11];0.

Available from: http://www.thelancet.com/article/S1473309921004795/fulltext.

[7] Duarte LF, Gálvez NMS, Iturriaga C, et al. Immune Profile and Clinical Outcome of Breakthrough Cases After Vaccination With an Inactivated SARS-CoV-2 Vaccine. Front Immunol [Internet]. 2021 [cited 2021 Oct 11];0:3884. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.742914/full.

[8] Jara A, Undurraga EA, González C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. https://doi.org/101056/NEJMoa2107715 [Internet]. 2021 [cited 2021 Oct 7];385:875–884. Available from: https://www.nejm.org/doi/full/10.1056/NEJMoa2107715.

[9] 91,5% de la población objetivo ha recibido la primera o única dosis de la vacuna contra SARS-CoV-2 - Ministerio de Salud - Gobierno de Chile [Internet]. [cited 2021 Oct 6]. Available from: https://www.minsal.cl/915-de-la-poblacion-objetivo-ha-recibido-la-primera-o-unica-dosis-de-la-vacuna-contra-sars-cov-2/.

[10] Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med 2021 277 [Internet]. 2021 [cited 2021 Oct 6];27:1205–1211. Available from: https://www.nature.com/articles/s41591-021-01377-8.

[11] Protection duration after vaccination or infection – Swiss National COVID-19 Science Task Force [Internet]. [cited 2021 Oct 6]. Available from: https://sciencetaskforce.ch/en/policy-brief/protection-duration-after-vaccination-or-infection/.

[12] Pan H, Wu Q, Zeng G, et al. Immunogenicity and safety of a third dose, and immune persistence of CoronaVac vaccine in healthy adults aged 18-59 years: interim results from a double-blind, randomized, placebo-controlled phase 2 clinical trial. medRxiv [Internet]. 2021 [cited 2021 Oct 6];2021.07.23.21261026. Available from: https://www.medrxiv.org/content/10.1101/2021.07.23.21261026v1.

[13] Cao Y, Yisimayi A, Bai Y, et al. Humoral immune response to circulating SARS-CoV-2 variants elicited by inactivated and RBD-subunit vaccines. Cell Res 2021 317 [Internet]. 2021 [cited 2021 Oct 8];31:732–741. Available from: https://www.nature.com/articles/s41422-021-00514-9.

[14] Melo-González F, Soto JA, González LA, et al. Recognition of Variants of Concern by Antibodies and T Cells Induced by a SARS-CoV-2 Inactivated Vaccine. Front Immunol [Internet]. 2021;12. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.747830/full.

[15] Depto Inmunizaciones D, De Prevención D, Control, Y Enfermedades S, et al. DOSIS DE REFUERZO EN LA CAMPAÑA DE VACUNACIÓN CONTRA SARS-COV-2 EN CHILE. 2021 [cited 2021 Oct 6]; Available from: https://www.minsal.cl/wp-.

[16] World medical association. World Medical Association declaration of Helsinki: Ethical principles for medical research involving human subjects [Internet]. JAMA - J. Am. Med. Assoc. JAMA; 2013 [cited 2021 Oct 6]. p. 2191–2194. Available from: https://pubmed.ncbi.nlm.nih.gov/24141714/.

[17] WHO/BS.2020.2403 Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody [Internet]. [cited 2021 Oct 8].

Available from: https://www.who.int/publications/m/item/WHO-BS-2020.2403.

[18] Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell. 2020;181:1489-1501.e15.

[19] Lumley SF, O'Donnell D, Stoesser NE, et al. Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers. https://doi.org/101056/NEJMoa2034545 [Internet]. 2020 [cited 2021 Oct 8];384:533–540. Available from: https://www.nejm.org/doi/10.1056/NEJMoa2034545.

[20] Sabino EC, Buss LF, Carvalho MPS, et al. Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. Lancet. 2021;397:452–455.

[21] Bar-On YM, Goldberg Y, Mandel M, et al. BNT162b2 vaccine booster dose protection: A nationwide study from Israel. medRxiv [Internet]. 2021 [cited 2021 Oct 8];2021.08.27.21262679. Available from: https://www.medrxiv.org/content/10.1101/2021.08.27.21262679v1.

[22] Wang K, Cao Y, Zhou Y, et al. A third dose of inactivated vaccine augments the potency, breadth, and duration of anamnestic responses against SARS-CoV-2. medRxiv [Internet]. 2021 [cited 2021 Oct 8];2021.09.02.21261735. Available from: https://www.medrxiv.org/content/10.1101/2021.09.02.21261735v1.

[23] Seyahi E, Bakhdiyarli G, Oztas M, et al. Antibody response to inactivated COVID-19 vaccine (CoronaVac) in immune-mediated diseases: a controlled study among hospital workers and elderly. Rheumatol Int 2021 418 [Internet]. 2021 [cited 2021 Oct 8];41:1429–1440. Available from:

https://link.springer.com/article/10.1007/s00296-021-04910-7.

[24] Widge AT, Rouphael NG, Jackson LA, et al. Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination. https://doi.org/101056/NEJMc2032195 [Internet]. 2020 [cited 2021 Oct 8];384:80–82. Available from: https://www.nejm.org/doi/full/10.1056/NEJMc2032195.

[25] Li M, Yang J, Wang L, et al. A booster dose is immunogenic and will be needed for older adults who have completed two doses vaccination with CoronaVac: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. medRxiv [Internet]. 2021 [cited 2021 Oct 8];2021.08.03.21261544. Available from: https://www.medrxiv.org/content/10.1101/2021.08.03.21261544v1.

[26] Mateus J, Dan JM, Zhang Z, et al. Low-dose mRNA-1273 COVID-19 vaccine generates durable memory enhanced by cross-reactive T cells. Science (80-) [Internet]. 2021 [cited 2021 Nov 11];374. Available from: https://www.science.org/doi/abs/10.1126/science.abj9853.

[27] Guerrera G, Picozza M, D'Orso S, et al. BNT162b2 vaccination induces durable SARS-CoV-2 specific T cells with a stem cell memory phenotype. Sci Immunol [Internet]. 2021 [cited 2021 Nov 11]; Available from: https://www.science.org/doi/abs/10.1126/sciimmunol.abl5344.

Figures

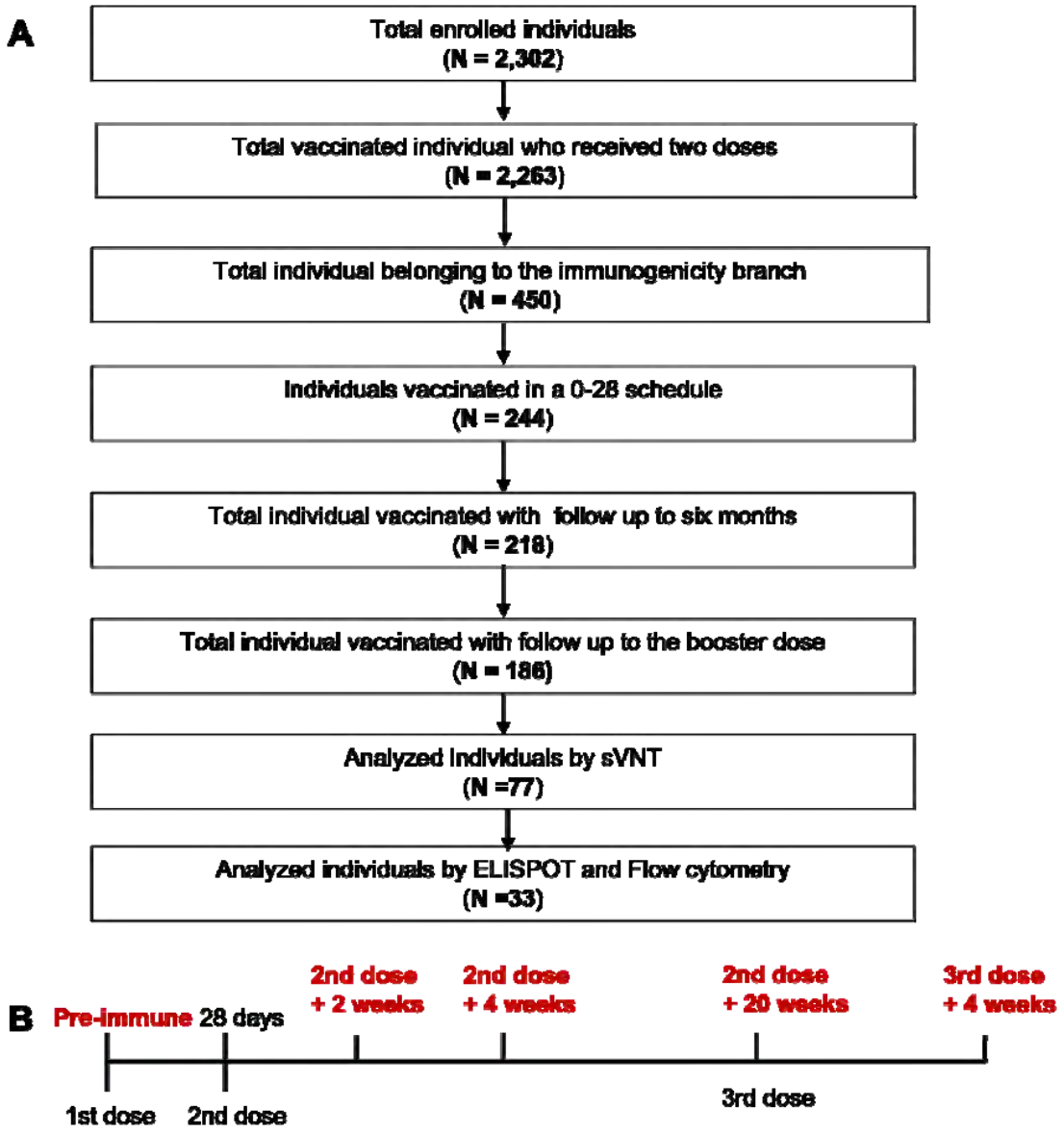


Figure 1: Study profile, enrolled volunteers and cohort included in this study on October 31st, 2021. 77 of the 450 vaccinated individuals belonging to the immunogenicity branch of the clinical trial conducted in Chile were selected of one of the centers of the study (the CL1-Marcoleta) for immunogenicity assays. B.

Timeline of 0–28-day schedule of vaccination and booster (third) dose immunization. Text in red denotes timepoints at which blood draws occurred.

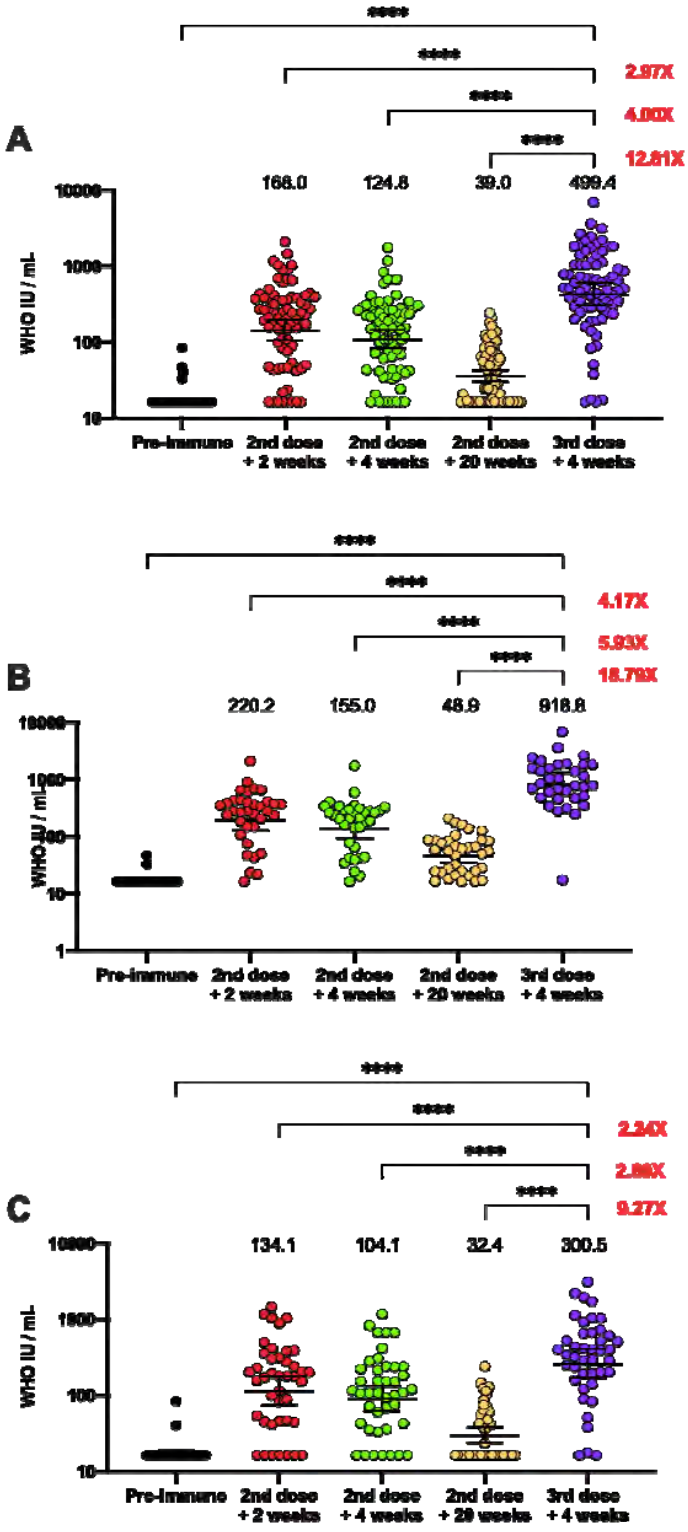

A
****
****
****
****
2.97X
4.00X
12.81X
168.0
124.8
39.0
499.4
WHO IU / mL
10000
1000
100
10
Pre-immune
2nd dose + 2 weeks
2nd dose + 4 weeks
2nd dose + 20 weeks
3rd dose + 4 weeks
B
****
****
****
****
4.17X
5.93X
18.78X
220.2
155.0
48.9
918.8
WHO IU / mL
1000
100
10
1
Pre-immune
2nd dose + 2 weeks
2nd dose + 4 weeks
2nd dose + 20 weeks
3rd dose + 4 weeks
C
****
****
****
****
2.34X
2.88X
9.27X
134.1
104.1
32.4
300.5
WHO IU / mL
1000
100
10
Pre-immune
2nd dose + 2 weeks
2nd dose + 4 weeks
2nd dose + 20 weeks
3rd dose + 4 weeks

Figure 2: Quantification of circulating antibodies inhibiting the interaction between the S1-RBD and hACE2 in volunteers that received the booster dose twenty weeks after the second dose, in a 0–28-day vaccination schedule. Inhibiting antibody titer is expressed as international units by using a WHO standard. Results were obtained from 77 volunteers (**A**), 36 of them were adults between 18-59 years old (**B**), and 41 of them were ≥ 60 years old (**C**). Data is represented as the logarithm of the WHO arbitrary units. Numbers above the bars show the Geometric Mean units (GMU), the error bars indicate the 95% CI, and the number at the right represents the fold increase of the GMU after the third dose compared with the respective time. A One-Way ANOVA test assessed statistical differences to compare all times against 3rd dose + four weeks. ****p<0.0001.

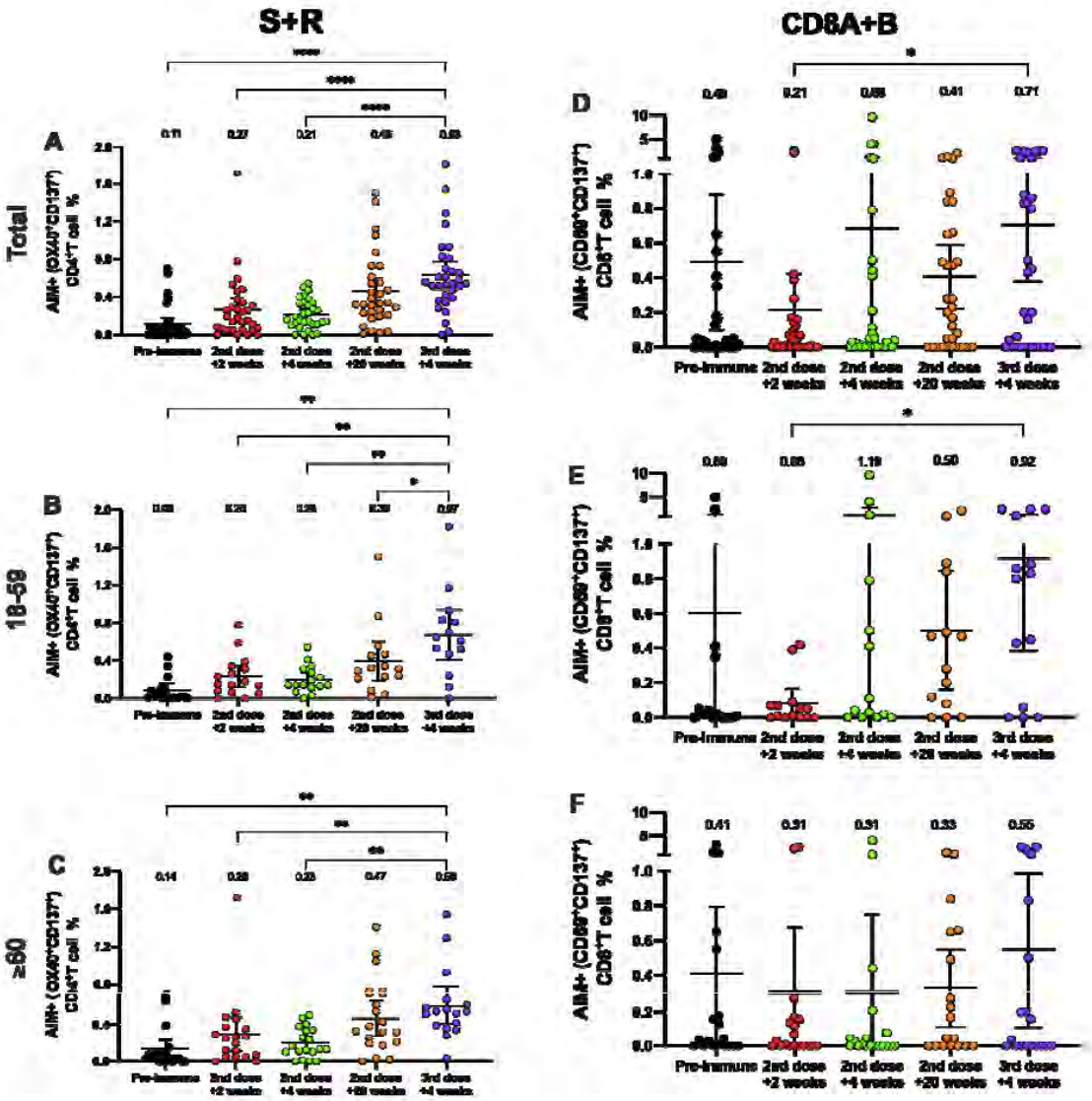


Figure 3: Changes in activation-induced markers (AIMs) expression in T cells through flow cytometry upon stimulation with Mega Pools of peptides derived from SARS-CoV-2 in volunteers immunized with CoronaVac with the booster dose, given twenty weeks after the second dose, in a 0–28-day vaccination schedule. The percentage of activated $CD4^+$ (AIM^+ [$OX40^+$, $CD137^+$]) and $CD8^+$ (AIM^+ [$CD69^+$, $CD137^+$]) T cells was determined by flow cytometry, upon

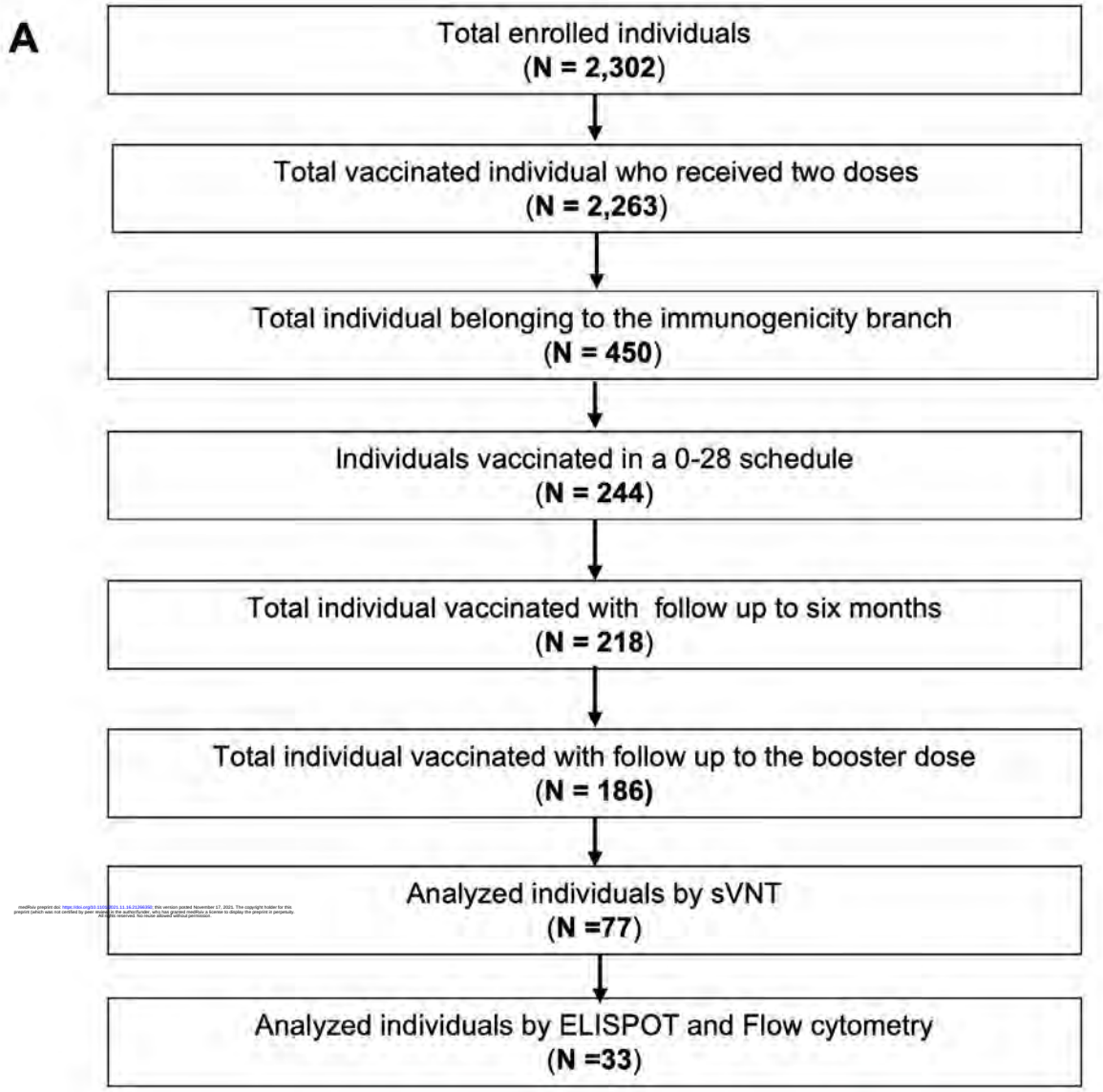
stimulation for 24h with MP-S+R (**A-C**) and MP-CD8A+B (**D-E**) in samples obtained at pre-immune, two weeks after the 2nd dose, four weeks after the 2nd dose, twenty weeks the 2nd dose, and four weeks after the 3rd dose. Results were obtained from a total of 33 volunteers (**A-D**), 14 were of them were adults between 18-59 years old (**B-E**), and 19 of them were ≥ 60 years old (**C-F**). Data shown represent means + 95%CI. Data from flow cytometry was normalized against DMSO and analyzed separately by One-way ANOVA with mixed effect analysis. *P<0.05; **p<0.005; ****p<0.0001.

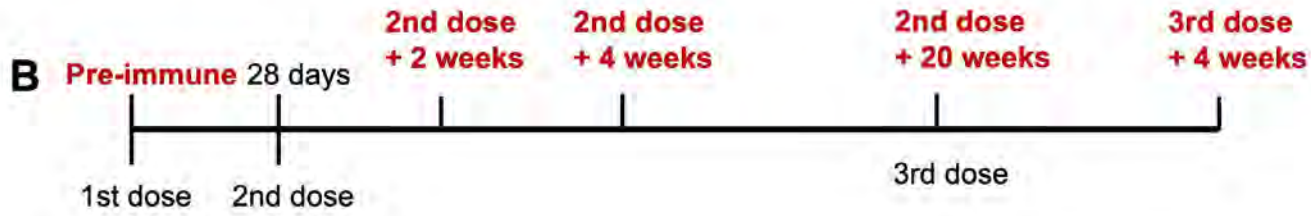
Table

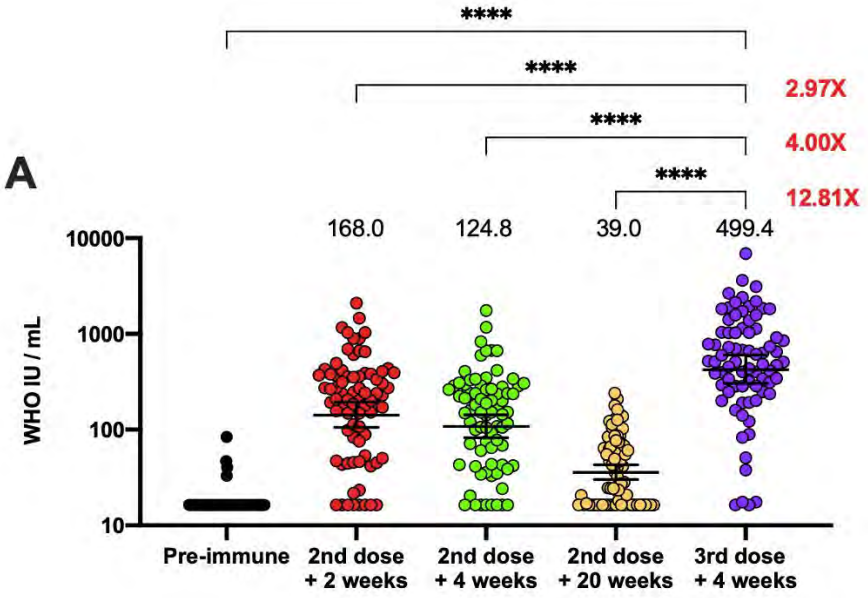
Table 1: Seropositivity rates, Geometric Median Titer (GMT), and Geometric Median Units (GMU) of circulating neutralizing antibodies against SARS-CoV-2 RBD.

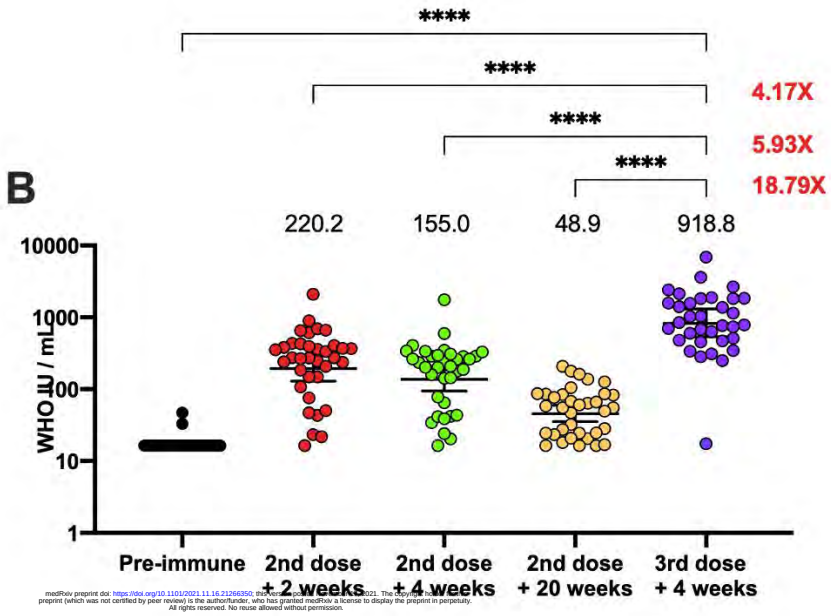
Age group	Indicators	2w after 2nd dose	4w after 2nd dose	5m after 2nd dose	4w after 2nd dose
Total Vaccine	Seropositivity n/N	72/77	73/77	38/77	75/77
	(%)	93.5	94.8	49.4	97.4
	GMU	168.0	124.8	39.0	499.4
	95% CI	126.8-222.5	96.3-161.7	32.4-47.0	370.6-673.0
	GMT	25.8	16.6	3.5	53.0
	95% CI	19.5-34.2	13.1-21.0	3.0-4.1	40.8-68.8
18-59	Seropositivity n/N	35/36	36/36	24/36	36/36
	(%)	97.2	97.2	66.7	100
	GMU	220.2	155.0	48.9	918.8
	95% CI	150.7-321.7	108.0-222.6	37.6-63.5	623.4-1354
	GMT	33.3	19.1	4.3	82.8
	95% CI	23.4-47.3	14.0-26.1	3.4-5.4	59.7-114.8
≥60	Seropositivity n/N	38/41	39/42	15/42	40/42
	(%)	90.5	92.9	35.7	95.2
	GMU	134.1	104.1	32.4	300.5
	95% CI	89.2-201.6	71.8-151.0	25.1-41.8	203.5-443.6
	GMT	20.8	14.7	2.9	36.5
	95% CI	13.6-31.9	10.3-21.0	2.4-3.5	25.3-52.7

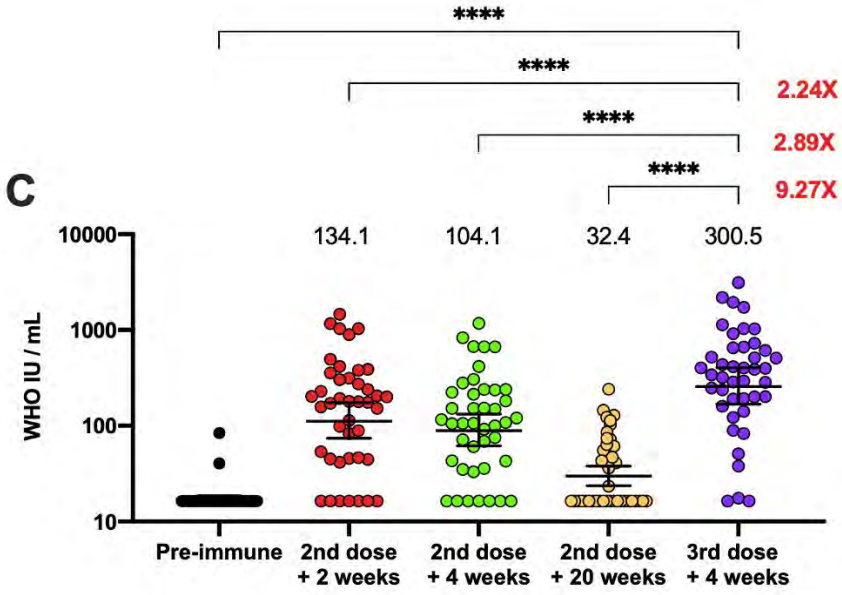
GMT: Geometric mean titer; GMU: Geometric mean units.

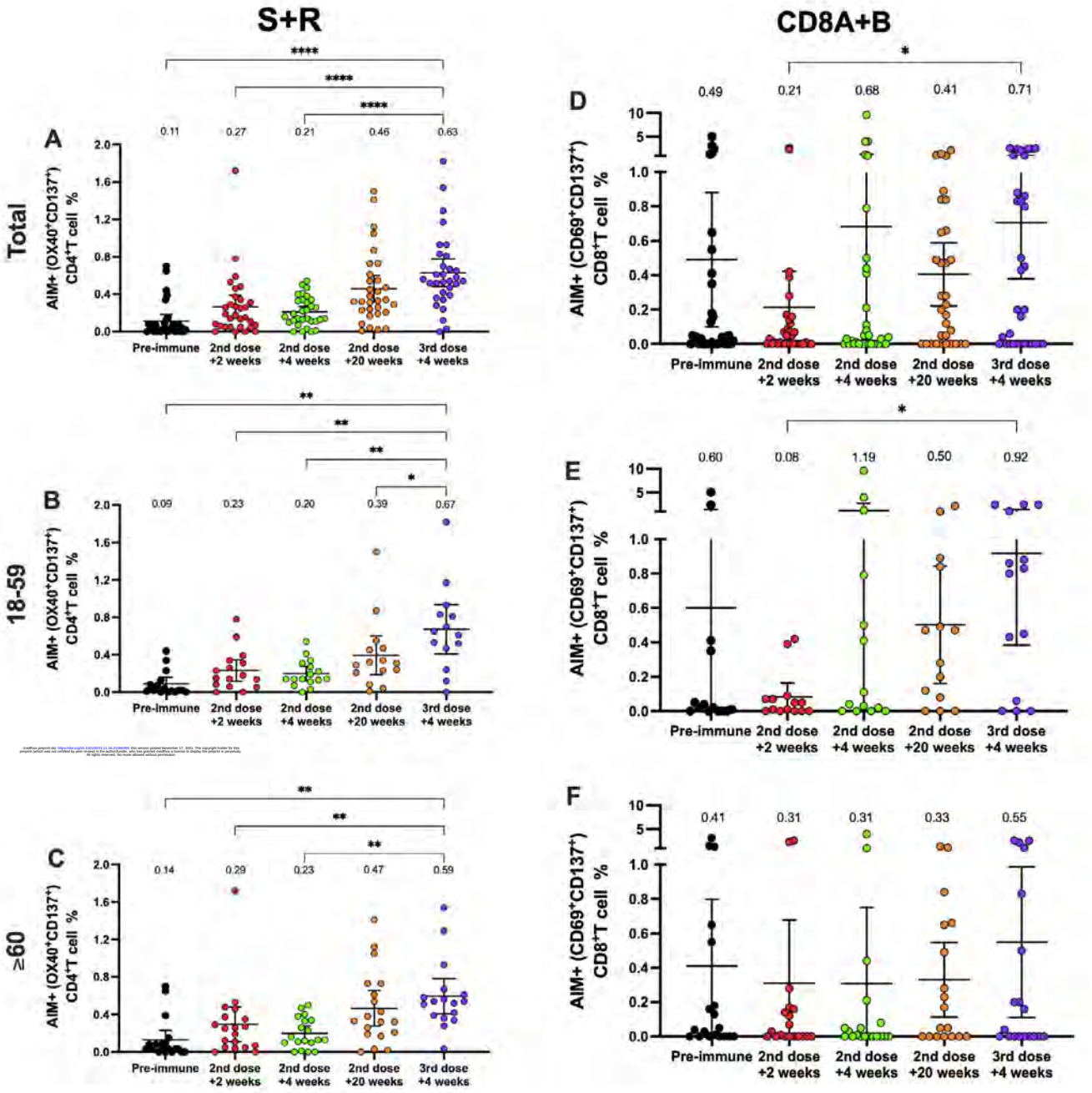











S+R
CD8A+B
Total
18-59
≥60
A
B
C
D
E
F
AIM+ (OX40+CD137+) CD4+T cell %
AIM+ (CD69+CD137+) CD8+T cell %
Pre-immune
2nd dose +2 weeks
2nd dose +4 weeks
2nd dose +20 weeks
3rd dose +4 weeks
0.11 0.27 0.21 0.46 0.63
0.09 0.23 0.20 0.39 0.67
0.14 0.29 0.23 0.47 0.59
0.49 0.21 0.68 0.41 0.71
0.60 0.08 1.19 0.50 0.92
0.41 0.31 0.31 0.33 0.55

7.8. Dose de reforço da CoronaVac eleva proteção contra Covid-19 para 80%, de acordo com governo chileno

O Ministério da Saúde do Chile anunciou que a aplicação de uma dose de reforço da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19 aumenta a eficácia do imunizante para 80,2%, e eleva a proteção contra hospitalizações de 84% para 88%. A pesquisa analisou o desempenho das três vacinas disponíveis no país (CoronaVac, Pfizer e AstraZeneca) na prevenção de casos e internações pela doença com base na campanha nacional de vacinação contra o SARS-CoV-2.

A principal conclusão é que o uso da dose de reforço da CoronaVac traz resultados muito semelhantes aos das demais vacinas, aumentando de forma considerável os níveis de eficácia contra a Covid-19 sintomática. Em relação à proteção contra casos em geral, a vacina da Pfizer-BioNTech aumentou o indicador de 56% para 90%, e a da AstraZeneca, de 56% para 93%. Já contra hospitalizações, a Pfizer-BioNTech causou um aumento de 84% para 87% na proteção, e a AstraZeneca, de 84% para 96,3%.

O estudo incluiu 4.785.749 pessoas imunizadas com o esquema completo de duas doses da CoronaVac, das quais 2.017.878 receberam a dose de reforço a partir de 11 de agosto. Destes, 1.506.154 tomaram a dose de reforço da AstraZeneca, 371.592 receberam a dose de reforço da Pfizer e 140.132, da CoronaVac. Todos os participantes são maiores de 16 anos, sem histórico de infecção por SARS-CoV-2.

De acordo com o infectologista e assessor do Ministério da Saúde do Chile Rafael Araos, o estudo revela que a decisão de aplicar a dose adicional para prevenir a Covid-19 foi acertada. "As três vacinas que usamos como reforço em pessoas que foram vacinadas com a CoronaVac têm um efeito superpoderoso", afirmou o médico. "Os resultados são robustos e sugerem que o efeito da dose de reforço, com qualquer vacina, é altamente eficaz na prevenção da Covid-19 e hospitalizações."

Publicado em: 7/10/2021

Ministerio de Salud

Gobierno de Chile

Immunization Campaign against SARS-CoV-2

Early estimates of the effectiveness of booster shots in Chile

Grupo para estudio de vacunas SARS-CoV-2 MINSAL (vCovid MINSAL)

October 2021

BACKGROUND

1 ENTREGA DE RESULTADOS ESTUDIO DOSIS DE REFUERZO

- Evidence suggest that neutralizing antibodies against SARS-CoV-2 induced by vaccines wane over time, which may decrease their effect against Covid-19 and it consequences.
- The longitudinal effectiveness assessments performed by the Chile Ministry of Health showed a sharp discrease in the effectiveness to prevent Covid-19, specifically within the group immunized with inactivated vaccines early on.
- International studies have shown that the combination of vaccines is safe and effectively increase levels of SARS-CoV-2 neutralizing antibodies.

2

DESIGN AND METHODS

- We analized a cohort of people that are affiliated with the National Health Fund (FONASA):
  - **Aged 16 years or older**
  - **No history of SARS-CoV-2 infection** (confirmed of probable Covid-19).
  - **That have already received CoronaVac** as a primary immunization.
- The effectiveness was estimated for each vaccine booster and focuses on preventing Covid-19 or Covid-19 related hospitalization. Outcomes were compared to the unvaccinated population.

3

DESIGN AND METHODS

- The effectivenes was estimated 14 days after receiving the booster shot with any of the available vaccines.
- The comparison groups consisted of people that received the booster dose or not. All the people contributed (person-days) to the non vaccinated group before starting their vaccination schedule.
- The results are independent from age, sex, place of residence, presence of comorbilities, nationality and income level.

4

RESULTS | CHARACTERISTICS OF THE COHORT

- The total sample was **11.201.635 people.**
- **500.145 cases of Covid-19.**
- The distribution of the covariates significantly differed between people immunized or not.

5

RESULTS | CHARACTERISTICS OF THE COHORT

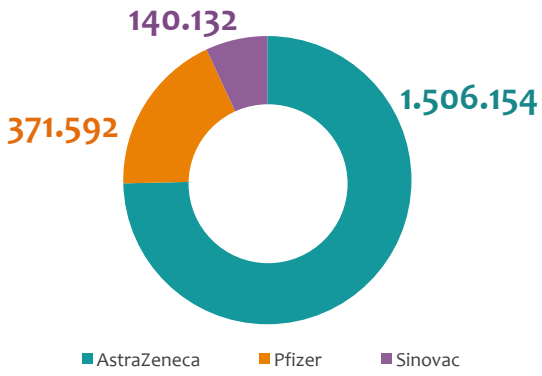
		Covid-19		Unvaccinated	Vaccinated			
					1 dose	2 doses	3 doses	
Characteristic	N (%)	N (row %)	p-value	N (row %)	N (row %)	N (%)	N (%)	p-value
Total	11,201,635 (100.0)	500,145 (4.5)	-	1,318,288 (11.7687)	719,263 (6.4211)	7,146,206 (63.7961)	2,017,878 (18.0141)	-
Region								
Arica	144,726 (1.3)	6,695 (4.6)	< 0.0001	21,489 (14.85)	10,608 (7.33)	92,597 (63.98)	20,032 (13.84)	< 0.0001
Tarapacá	200,869 (1.8)	8,828 (4.4)		32,257 (16.06)	12,694 (6.32)	131,619 (65.52)	24,299 (12.1)	
Antofagasta	329,632 (2.9)	10,659 (3.2)		43,639 (13.24)	24,239 (7.353)	218,761 (66.37)	42,993 (13.04)	
Atacama	191,906 (1.7)	5,991 (3.1)		23,938 (12.47)	12,995 (6.772)	127,012 (66.18)	27,961 (14.57)	
Coquimbo	531,115 (4.7)	17,518 (3.3)		59,364 (11.18)	34,141 (6.428)	356,775 (67.17)	80,835 (15.22)	
Valparaíso	1,212,562 (11)	44,364 (3.7)		150,740 (12.43)	71,947 (5.933)	747,759 (61.67)	242,116 (19.97)	
Metropolitana	4,098,579 (37)	184,233 (4.5)		505,690 (12.34)	267,377 (6.524)	2,530,109 (61.73)	795,403 (19.41)	
L.G.B. O'Higgins	629,292 (5.6)	24,266 (3.9)		60,130 (9.555)	33,564 (5.334)	428,027 (68.02)	107,571 (17.09)	
Maule	762,796 (6.8)	38,424 (5)		73,288 (9.608)	45,159 (5.92)	508,071 (66.61)	136,278 (17.87)	
Ñuble	348,527 (3.1)	14,062 (4)		32,392 (9.294)	16,905 (4.85)	236,007 (67.72)	63,223 (18.14)	
Biobío	1,054,437 (9.4)	54,087 (5.1)		101,632 (9.639)	61,591 (5.841)	686,255 (65.08)	204,959 (19.44)	
Araucanía	683,250 (6.1)	41,357 (6.1)		86,887 (12.72)	45,239 (6.621)	439,443 (64.32)	111,681 (16.35)	
Los Ríos	273,268 (2.4)	17,420 (6.4)		32,246 (11.8)	17,606 (6.443)	179,344 (65.63)	44,072 (16.13)	
Los Lagos	584,765 (5.2)	25,950 (4.4)		76,188 (13.03)	47,534 (8.129)	372,198 (63.65)	88,845 (15.19)	
Aysén	61,227 (0.55)	2,199 (3.6)		7,143 (11.67)	6,980 (11.4)	38,864 (63.48)	8,240 (13.46)	
Magallanes	94,684 (0.85)	4,092 (4.3)		11,265 (11.9)	10,684 (11.28)	53,365 (56.36)	19,370 (20.46)	

6

RESULTS

4.785.749 people immunized with CoronaVac were included.

2.017.878 received one booster shot.



7

RESULTS

INCREASED EFFECTIVINESS AGAINST COVID-19

14 DAYS AFTER THE BOOSTER SHOT

Booster shot CoronaVac	Booster shot Pfizer-BioNTech	Booster shot AstraZeneca
56% to 80%	56% to 90%	56% to 93%

8

RESULTS

INCREASED EFFECTIVINESS AGAINST HOSPITALIZATION

14 DAYS AFTER THE BOOSTER SHOT

Booster shot CoronaVac	Booster shot Pfizer-BioNTech	Booster shot AstraZeneca
84% to 88%	84% to 87%	84% to 96%

9

CONCLUSION

- The three vaccines used as a booster notably **increased the effectiveness against Covid-19 and related hospitalizations.**
- These results support the decission to initiate a boosting program among people immunized with inactivated vaccines.

10


Ministerio de Salud
Gobierno de Chile
#YO ME VACUNO
gob.cl/yomevacuno

8. Raros efeitos adversos

8.1. CoronaVac apresenta eventos adversos em menos de 1% das doses aplicadas em adultos no Brasil e mostra-se altamente segura para vacinados

A CoronaVac, vacina desenvolvida pelo Instituto Butantan em parceria com a farmacêutica chinesa Sinovac, apresentou eventos adversos em menos de 1% das doses aplicadas em maiores de 18 anos no Brasil. Os dados são da Farmacovigilância do Instituto e são mais um indicador de que a vacina é altamente segura e muito pouco reatogênica.

"A CoronaVac tem um perfil de segurança muito bom e vimos isso pela proporção entre o número de vacinados e o total de eventos adversos notificados.", explica a pesquisadora científica e responsável pela Farmacovigilância do Instituto Butantan, Vera Gattás.

Os dados da vida real reforçam os estudos realizados com a CoronaVac, que indicam que a vacina é altamente segura para este público vacinado.

"Em números, isso significa que em cerca de 130 milhões de doses aplicadas no país, houve em torno de mil casos notificados de eventos adversos graves e 7.000 eventos adversos não graves, em ambos não chegam a 1% das doses aplicadas", informa Vera.

Os estudos com crianças a partir de 3 anos também apresentaram bons resultados de segurança. As reações mais comuns foram locais, como dor, inchaço e vermelhidão no local da aplicação.

"Verificamos ainda que os benefícios da vacinação com CoronaVac superam muito os seus riscos de eventos adversos", reitera a líder da farmacovigilância do Butantan.

Diferença entre evento e reação adversa

"Toda reação adversa é um evento adverso, mas nem todo evento adverso é uma reação adversa, e cabe à Farmacovigilância fazer esta distinção", pondera Vera.

Eventos adversos são quaisquer ocorrências que acontecem após o uso de algum medicamento, soro ou vacina, podendo ter ou não relação com o produto usado.

Já as **reações adversas** são quaisquer sintomas ou eventos prejudiciais que ocorrem após o uso do medicamento, soro ou vacina, com alguma possibilidade de terem sido causadas pelo uso de algum destes produtos.

Entre os eventos adversos notificados à Farmacovigilância do Butantan, a maioria foi classificada como leve (grau 1) ou moderado (grau 2). São eventos esperados como dor e no local da aplicação, febre e dor no corpo que se resolvem nos primeiros dias após a aplicação.

Como é feita a farmacovigilância

O monitoramento dos relatos de eventos adversos é a base da farmacovigilância, responsável por continuar o trabalho de averiguação da segurança, eficácia e qualidade das vacinas após chegar ao braço das pessoas. Este acompanhamento continua sendo feito pelo fabricante após a imunização como forma de prevenir e detectar alterações no perfil de segurança e na ocorrência e frequência dos eventos adversos.

No Butantan, o monitoramento da CoronaVac e de outras vacinas, medicamentos e soros feito pela equipe de Farmacovigilância. As notificações chegam por meio do Serviço de Atendimento ao Consumidor (SAC) (veja os contatos abaixo) e dos canais de comunicação oficiais do Instituto Butantan.

"Estamos sempre atentos e de portas abertas para receber informações que possam indicar efeitos adversos da vacinação ou outros medicamentos produzidos no Butantan. Para isso, contamos com diversos canais de comunicação de onde acumulamos dados e fazemos um panorama que tem como objetivo monitorar os produtos fabricados visando fornecer informações para a avaliação da segurança, eficácia e qualidade do produto", detalha a pesquisadora.

Segundo Vera, nem sempre fica claro para a população que é pela Farmacovigilância que se mantém a garantia de segurança, eficácia e qualidade das vacinas usadas. E que, em caso de quaisquer sintomas após a vacinação, eles devem ser reportados para que se tenha um controle.

"Se após a análise da Farmacovigilância se demonstre que o relato não tem necessariamente uma relação com o uso da vacina, mesmo assim é importante relatá-lo para a informação ser enviada para as áreas técnicas analisarem", conclui a pesquisadora.

Como acionar a Farmacovigilância do Butantan

Serviço de Atendimento
ao Consumidor

Telefone: **0800 701 2850**
e-mail: **sac@butantan.gov.br**
E-mail da Farmacovigilância:
farmacovigilancia@butantan.gov.br

Publicado em: 3/6/2022

8.2. CoronaVac é segura e apresenta menos efeitos adversos graves, mostra estudo da Malásia

Um estudo realizado na Malásia demonstrou mais uma vez o elevado perfil de segurança da CoronaVac e a baixa ocorrência de reações adversas após a imunização. Entre as 9,3 milhões de pessoas que tomaram a vacina no país, não foram registrados eventos adversos trombóticos graves. O trabalho foi publicado na revista Vaccine e conduzido por pesquisadores do Instituto Nacional de Saúde da Malásia, da Agência Nacional de Regulação Farmacêutica e de sete hospitais do país.

Até o final de agosto de 2021, 35,2 milhões de doses de vacinas contra Covid-19 haviam sido administradas em mais de 20 milhões de pessoas na Malásia, sendo que a CoronaVac foi o imunizante mais utilizado, com 17 milhões de doses (48% do total), seguida por Pfizer (44%) e AstraZeneca (8%). Cerca de 78% dos 9,3 milhões de indivíduos vacinados com a CoronaVac completaram as duas doses da vacina.

A CoronaVac não foi associada a nenhum efeito adverso trombótico grave, mas foi relacionada a um pequeno aumento no risco de arritmia. Já nos indivíduos vacinados com imunizante de RNA mensageiro, houve leve aumento do risco de tromboembolismo venoso, arritmia e convulsão. Naqueles que receberam a vacina de vetor viral, foi significativo o aumento do risco de trombocitopenia e tromboembolismo venoso.

No entanto, os pesquisadores não registraram risco de miocardite, pericardite, paralisia de Bell, acidente vascular cerebral e infarto do miocárdio nos 21 dias após a administração de qualquer uma das vacinas. O número absoluto de eventos adversos foi baixo para todas as vacinas. "Vale ressaltar que os benefícios gerais dos imunizantes superam os potenciais riscos e essas informações devem ser interpretadas em conjunto com os dados de efetividade das vacinas", dizem os autores do estudo.

CoronaVac é segura em pacientes com risco aumentado para trombose

Uma pesquisa conduzida pela Faculdade de Medicina da Universidade de São Paulo (FMUSP) com indivíduos com alto risco de trombose, portadores de uma doença autoimune chamada síndrome antifosfolípide, mostrou que a CoronaVac é uma vacina segura para esse público, não influenciando na doença. Além disso, o imunizante foi altamente imunogênico, induzindo produção de anticorpos em mais de 80% dos pacientes.

Publicado em: 3/6/2022

Vaccine xxx (xxxx) xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Risk of serious adverse events after the BNT162b2, CoronaVac, and ChAdOx1 vaccines in Malaysia: A self-controlled case series study

Norazida Ab Rahman [a,*], Ming Tsuey Lim [a], Fei Yee Lee [a], Sing Chet Lee [b], Azuana Ramli [b], Siti Nurhafizah Saharudin [c], Teck Long King [d], Emelyne Bani Anak Jam [e], Nor Aliya Ayub [f], Raj Kumar Sevalingam [f], Rashidah Bahari [g], Nor Nadziroh Ibrahim [g], Fatihah Mahmud [h], Sheamini Sivasampu [a], Kalaiarasu M Peariasamy [a], for the SAFECOVAC study group

[a] Institute for Clinical Research, National Institutes of Health, Selangor, Malaysia
[b] National Pharmaceutical Regulatory Agency, Selangor, Malaysia
[c] Clinical Research Centre, Shah Alam Hospital, Selangor, Malaysia
[d] Clinical Research Centre, Sarawak General Hospital, Sarawak, Malaysia
[e] Clinical Research Centre, Queen Elizabeth II Hospital, Sabah, Malaysia
[f] Clinical Research Centre, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia
[g] Clinical Research Centre, Putrajaya Hospital, Putrajaya, Malaysia
[h] Clinical Research Centre, Tengku Ampuan Afzan Hospital, Pahang, Malaysia

ARTICLE INFO

Article history:
Received 14 March 2022
Received in revised form 10 May 2022
Accepted 12 May 2022
Available online xxxx

Keywords:
COVID-19 vaccines
Adverse events of special interest
Safety
Self-controlled case series

ABSTRACT

Background: Rapid deployment of COVID-19 vaccines is challenging for safety surveillance, especially on adverse events of special interest (AESIs) that were not identified during the pre-licensure studies. This study evaluated the risk of hospitalisations for predefined diagnoses among the vaccinated population in Malaysia.

Methods: Hospital admissions for selected diagnoses between 1 February 2021 and 30 September 2021 were linked to the national COVID-19 immunisation register. We conducted self-controlled case-series study by identifying individuals who received COVID-19 vaccine and diagnosis of thrombocytopenia, venous thromboembolism, myocardial infarction, myocarditis/pericarditis, arrhythmia, stroke, Bell's Palsy, and convulsion/seizure. The incidence of events was assessed in risk period of 21 days postvaccination relative to the control period. We used conditional Poisson regression to calculate the incidence rate ratio (IRR) and 95% confidence interval (CI) with adjustment for calendar period.

Results: There was no increase in the risk for myocarditis/pericarditis, Bell's Palsy, stroke, and myocardial infarction in the 21 days following either dose of BNT162b2, CoronaVac, and ChAdOx1 vaccines. A small increased risk of venous thromboembolism (IRR 1.24; 95% CI 1.02, 1.49), arrhythmia (IRR 1.16, 95% CI 1.07, 1.26), and convulsion/seizure (IRR 1.26; 95% CI 1.07, 1.48) was observed among BNT162b2 recipients. No association between CoronaVac vaccine was found with all events except arrhythmia (IRR 1.15; 95% CI 1.01, 1.30). ChAdOx1 vaccine was associated with an increased risk of thrombocytopenia (IRR 2.67; 95% CI 1.21, 5.89) and venous thromboembolism (IRR 2.22; 95% CI 1.17, 4.21).

Conclusion: This study shows acceptable safety profiles of COVID-19 vaccines among recipients of BNT162b2, CoronaVac, and ChAdOx1 vaccines. This information can be used together with effectiveness data for risk-benefit analysis of the vaccination program. Further surveillance with more data is required to assess AESIs following COVID-19 vaccination in short- and long-term.

1. Introduction

The administration of COVID-19 vaccines is viewed as the most promising approach to curb the pandemic. By the end of 2021, nearly nine billion doses of COVID-19 vaccines had been administered worldwide [1]. The vaccines have been proven to be safe and effective in clinical trials. Yet, as with the initiation of any new

* Corresponding author at: Institute for Clinical Research, Block B4, National Institutes of Health, No 1, Jalan Setia Murni U13/52, 40170 Shah Alam, Selangor, Malaysia.
E-mail address: norazida@crc.gov.my (N. Ab Rahman).

https://doi.org/10.1016/j.vaccine.2022.05.075
0264-410X/

drugs, information on long-term safety and effectiveness is still being gathered during the post-marketing phase [2]. Rapid deployment of COVID-19 vaccines on a mass scale poses challenges for monitoring vaccine safety, including those not identified during the pre-licensure studies. Adverse effects following immunisation (AEFI) signal detection have primarily relied on passive surveillance reporting; however, it is often limited by incomplete information or under-reporting [3,4]. As such, enhancement of the type and scope of vaccine monitoring activities, including the conduct of active surveillance activities and well-designed observational study could refine the collecting and processing of information on COVID-19 vaccine safety [5].

Variations in the safety profile between different vaccine platform are to be expected as the different mechanism was used to trigger an immune response. mRNA vaccines (BNT162b2 and mRNA-1273) and adenoviral vector vaccines (ChAdOx1, Ad26.COV2.S) have been most extensively studied due to their high prevalence in western countries. Little information is currently available on the inactivated vaccine (CoronaVac, BBIBP-CorV) that has significant uptake globally [6]. Since the initiation of the COVID-19 immunisation program in Malaysia in late February 2021, 65% of the adult population has been vaccinated by 31 August 2021 [7]. Diverse vaccine portfolios were administered to the population where the majority were inoculated with BNT162b2, CoronaVac, or ChAdOx1 vaccines while a smaller proportion received other vaccines including CanSino, Sinopharm BBIBP-CorV, Ad26.COV.S. With the different types of vaccines administered, questions are arising on the comparability of these vaccines, adverse events, and the required period of monitoring.

We established a case-based surveillance approach for adverse events of special interest (AESI) following COVID-19 vaccination using routinely collected administrative databases and health records. This project was initiated to improve the capacity of COVID-19 vaccine safety monitoring in the country and complement the current adverse events monitoring through the national passive surveillance system [8]. We evaluated the risk of serious adverse events potentially associated with COVID-19 vaccines by focusing on cases that require hospitalisation among the vaccinated population in Malaysia. In this paper, we present the interim analysis of the ongoing study that covers the first half period of the COVID-19 immunisation roll-out in Malaysia that evaluates the occurrence of AESIs among the vaccinated population. We compared the rate of pre-specified AESI between different vaccine platforms and evaluated whether there was an increased risk of events after COVID-19 vaccination.

2. Materials and methods

2.1. Data sources

Data on vaccination was retrieved from the Malaysia Vaccine Administration System (MyVAS) database that was launched to manage vaccination records for the National COVID-19 Immunisation programme in Malaysia (Supplement 1). The database includes information on vaccine brand, dates, and doses as well as demographic details and self-reported comorbidity for the vaccinated population in Malaysia.

Hospital admission data were obtained from the Malaysian Data Warehouse (MyHDW), a national health data repository that collects data from public and private hospitals in Malaysia. This data included diagnoses coded according to the International Classification of Disease (ICD-10), admission and discharge dates, and discharge status which was monitored and validated by the Health Informatics Centre, Ministry of Health Malaysia [9]. Between February and September 2021, data from 216 public and private hospitals were available for analysis. Outcome data were also obtained from two other sources: sentinel surveillance sites and the national pharmacovigilance database. This allowed for a more immediate identification of eligible cases to account for the lag in data accrual due to delays in submission to the central repository by the data providers. Eight public tertiary hospitals across Malaysia were selected as sentinel surveillance sites for this study. Eligible cases were sourced directly from locally held records at these hospitals i.e., hospital discharge database and cases were identified from ICD to 10 coded diagnoses. The pharmacovigilance database was used to identify outcome events from spontaneous AEFI reports submitted by healthcare professionals, pharmaceutical companies, or consumers. Only cases that require hospitalisation were included for analysis. Outcome data from all sources were cross-linked to check for overlapping records.

Data on COVID-19 confirmed cases were retrieved from the national COVID-19 surveillance system. This dataset was used to determine COVID-19 diagnosis status within the study population based on the infection date.

All datasets were linked using unique resident identification numbers to establish the cohort for this study. Record linkage was conducted using deterministic matching of identifiers and de-identified data was used for analysis.

2.2. Study design

We used self-controlled case-series (SCCS) study design to examine the associations between COVID-19 vaccination and outcome events by comparing the incidence of events across risk periods relative to the control period. SCCS design employs within-person comparison and time-invariant confounders (e.g., sex, ethnicity, lifestyle, chronic diseases) are self-adjusted [10].

2.3. Study population

The study population comprised individuals who received at least one dose of the COVID-19 vaccine and were admitted to hospitals with the outcome of interest between 1 February to 30 September 2021. Only the first event within this period was included in the analysis. Those who had records of hospital admissions for the same diagnosis in the two years before the study period were excluded. We also excluded individuals who were COVID-19 positive (i) during admission and (ii) in the 30-day interval before. To allow for sufficient follow-up time to capture the outcome, the cohort was restricted to vaccine doses administered up to 31 August 2021.

2.4. Outcome

Outcomes were hospital admission associated with the pre-selected diagnoses of interest: thrombocytopenia, venous thromboembolism, stroke, myocardial infarction, myocarditis/pericarditis, cardiac arrhythmia, Bell's Palsy, and convulsion/seizures. Cases were identified using the ICD-10 diagnosis code in the diagnoses fields or cause of death recorded. The list of ICD-10 codes for each outcome is available in Supplement 2. The event date was the earliest date of hospital admission.

2.5. Exposure

Exposure was defined as receipt of one or more doses of COVID-19 vaccine. The date of vaccination was used as the date of exposure and individuals were classified according to the type of vaccine administered.

2.6. Statistical analysis

Characteristics of the study population (vaccinated individuals who developed the outcomes of interest) and the outcome events were described descriptively. The number of cases was tabulated by weeks since vaccination to describe event distribution. The absolute rate of events was calculated by dividing the total number of events by total doses administered and total persons vaccinated.

The SCCS method was used to investigate the association between outcome events and vaccination. Each patient follow-up time is divided into several periods: an exposed period (risk period) and an unexposed period (control period) (Supplement 3). The risk period comprised of 1 to 21 days after vaccination. The 21-day duration was defined based on literature and vaccine dose interval [11]. Given repeat exposures in which individuals may receive more than one dose of vaccine during the observation period, the risk period was defined as 21-day duration after any vaccine dose. The day of vaccination was defined as day 0 and included as a separate period. The 14 days before administration of the first vaccine dose was considered a pre-risk period since an event during this period is likely to affect the likelihood of receiving vaccination. All other observation times outside of these periods between 1 February 2021 and 30 September 2021 were defined as the control period.

The incidence rate ratio (IRR) of events in the risk period (exposed) and control period (unexposed) were calculated with the corresponding 95% confidence interval (CI). Each outcome was modelled separately using conditional Poisson regression with an offset of the length of risk periods. The models were adjusted for the month in the observation period to account for potential factors associated with calendar time. A 95% CI that did not include one indicates statistical significance. Using a risk period of 21 days, an observation period from 1 February to 30 September 2021, and IRR of 2.0 and 1.5, the sample size needed was 151 and 500, respectively, to estimate results with 80% power at 5% significant level. Reducing the IRR or risk period increases sample size requirements.

The rate ratio was also estimated for dose-effect where each dose of COVID-19 vaccine was regarded as a separate exposure and the risk periods were segmented by dose. Subgroup analysis was performed by age (<60 versus $\geq$ 60 years old) and sex of the patients to offset the risk of complications resulting from age and sex differences. Several measures were undertaken to assess assumptions of the SCCS method. To account for event dependent observation periods where the event may increase the risk of mortality and patient died before the end of observation, sensitivity analysis was carried out by excluding fatal events. Event dependent exposures were circumvented by including a pre-exposure risk period of 14 days. Another assumption of the SCCS method is that events must be independent of one another; therefore, analysis was restricted to only the first event.

Data were processed and analysed using STATA SE 15.0.

2.7. Ethical approval

This study was part of the project "Case-based monitoring of adverse events following COVID-19 vaccination – SAFECOVAC" that received approval from the Medical Research and Ethics Committee, Ministry of Health Malaysia (NMRR-21-322-59745) which include a waiver of informed consent due to the use of secondary data for this research.

3. Results

By 31 August 2021, up to 35,201,509 doses of COVID-19 vaccines were administered to over 20 million individuals in Malaysia. BNT162b2 and CoronaVac vaccines accounted for 44% and 48%, respectively, 8% were ChAdOx1 while other vaccines accounted for less than 1%. Of individuals who had received at least one dose of the vaccine, 51% were aged 18–39 years and 16% were 60 years and older. Only 38% of individuals vaccinated with ChAdOx1 had completed two doses during this period, compared to over 70% for both BNT162b2 and CoronaVac recipients. The total number of doses administered and vaccinated persons by sex and age are shown in Supplement 4.

3.1. Event characteristics

Fig. 1 shows an overview of patients included in the primary analysis by the individual outcome events for hospital admissions between 1 February 2021 and 30 September 2021 among the vaccinated population. The number of events ranged from 87 (myocarditis/pericarditis) to 10,487 (ischaemic stroke). We presented results for BNT162b2, CoronaVac, and ChAdOx1 vaccines as the events with other vaccine types were too small. Events that occurred in the first three weeks after vaccination was approximately 45% of all events observed in the postvaccination period (Supplement 5); the event distribution pattern was similarly observed with all three vaccines.

Table 1 shows the characteristics of events and demographics of patients who had hospital admissions for the outcome events in the 21 days following vaccination. More events were recorded after the first dose of vaccine than the second dose. Patients aged 60 years and older accounted for more than half of the cases, but the mean age was younger for those who had myocarditis/pericarditis (43.6 years), Bell's Palsy (50.1 years), and convulsion/-seizures (50.6 years). There was a preponderance of males among those with myocardial infarction (75%), stroke (60%), and myocarditis/pericarditis (60%). Nearly 50% of the patients who had myocardial infarction, arrhythmia, and stroke were reported to have hypertension.

3.2. Absolute rate of events

Table 2 shows the absolute rate for each event by vaccine type. Ischaemic stroke was the most frequent and the absolute event rate within 21 days of any vaccination was between 33 and 115 cases per million doses administered. This was followed by myocardial infarction (28 to 90 cases per million doses) and arrhythmia (27 to 95 cases per million doses). The event rate for others was much lower at less than 30 cases per million doses for each diagnosis. The absolute event rates appeared to be slightly higher among BNT162b2 vaccine recipients compared to CoronaVac and ChAdOx1 vaccine recipients for most events.

3.3. Incidence rate ratio of events

Fig. 2 shows the incidence rate ratio of events in the 21-day risk period following either dose of BNT162b2, CoronaVac, and ChAdOx1 vaccines compared to the control period. IRR estimates accounting for the vaccine dose effect are summarised in Table 3 and the IRR in all subintervals within the risk and control periods are shown in Supplement 6.

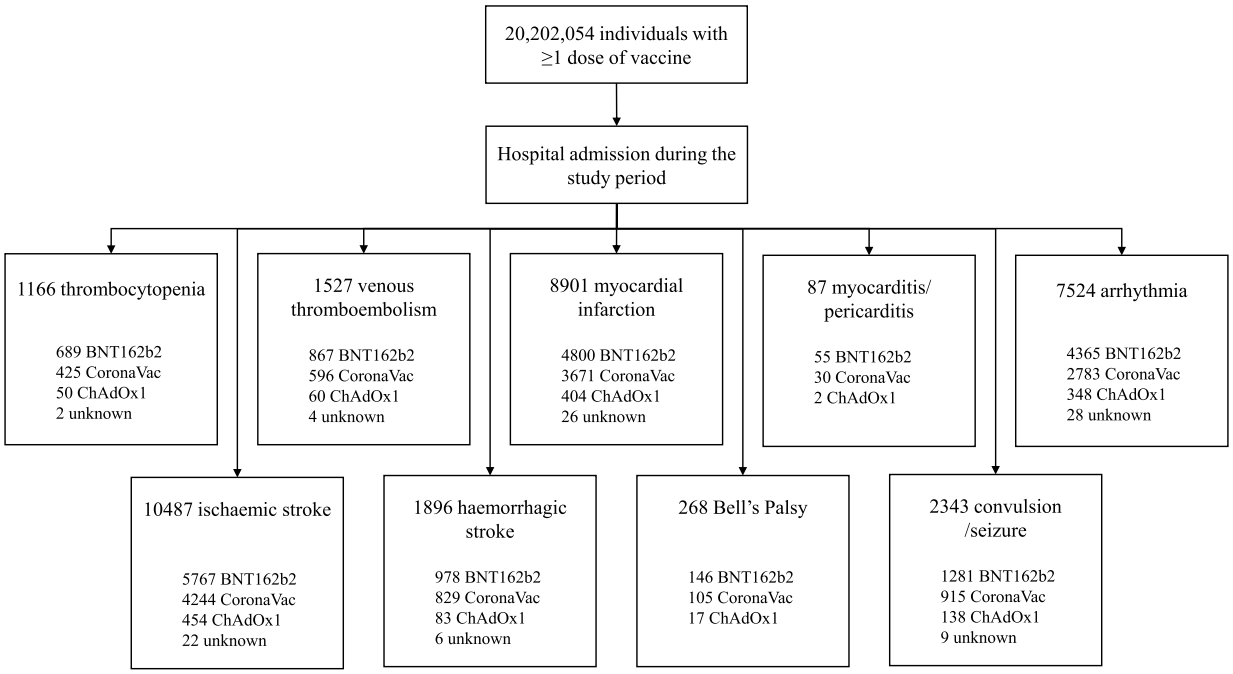


Fig. 1. Flow diagram of study population. The study period is from 1 February 2021 to 30 September 2021. Unknown refers to those whose vaccine type was not available.

In the 21 days following either vaccine dose, no significant increased risk was found for myocarditis/pericarditis, Bell's Palsy, stroke, and myocardial infarction in all vaccine platforms (Fig. 2). Among persons receiving BNT162b2 vaccine, the IRRs showed a slight elevation in the risk of venous thromboembolism, arrhythmia, and convulsion/seizure. In the analysis by vaccine dose, a significant association for venous thromboembolism was observed following the first dose (IRR 1.34; 95% CI 1.07, 1.67) whereas the risk of convulsion/seizure was significant after the second dose (IRR 1.39; 95% CI 1.12, 1.72) of BNT162b2 vaccine (Table 3). Overall estimates showed no association between CoronaVac vaccine and all outcomes, except arrhythmia. When the analysis was conducted by vaccine dose number, the risk appears to be slightly increased for myocardial infarction (IRR 1.16; 95% CI 1.02, 1.32), arrhythmia (IRR 1.18; 95% CI 1.02, 1.37), and haemorrhagic stroke (IRR 1.31; 95% CI 1.02, 1.68) after the first dose of CoronaVac vaccine. ChAdOx1 vaccine showed a significant association for several outcome events including thrombocytopenia and venous thromboembolism, but the number of events was smaller in this group resulting in a wider confidence interval.

3.4. Subgroup analyses by age group and sex and sensitivity analysis

The IRR estimates for subgroup analyses by age and sex are shown in Supplement 7. Stratification by sex showed the risk of venous thromboembolism and arrhythmia among BNT162b2 and CoronaVac recipients were higher in males. On the other hand, the risk of ischaemic stroke was significant among female CoronaVac recipients. Age effect was observed where patients aged ≥ 60 years have a higher risk of stroke, arrhythmia, and convulsion/seizure than their younger counterparts. No significant association was observed for any outcome among patients younger than 60 years old.

In a sensitivity analysis, cases of fatal admissions were excluded. Results of this additional analysis were consistent with those of the main findings which suggest a minimal bias for event dependent observation period (data not shown).

4. Discussion

This analysis of a population-based study covering over 20 million vaccinated individuals in Malaysia shows the risk of hospitalisation for predefined diagnoses among those vaccinated with BNT162b2, CoronaVac, and ChAdOx1 vaccines. We found that majority of events that occurred post-exposure were largely concentrated during the first three weeks after vaccination. We evaluated the relative risk of outcome events in 21 days following vaccination compared to the control period and found no significant increased risk for most events. Despite the statistically significant association for several events after vaccination, the point estimate of incidence rate ratios and lower limit of the 95% confidence interval were close to 1. The absolute number of events was low in all three vaccines.

We did not find a significant association between COVID-19 vaccination and cardiovascular complications, except arrhythmia. Cases of increased or irregular heart rate could be a physiological or stress-related response to vaccination [12,13] which could explain the findings where most events occurred during 1–7 days after vaccination. Arrhythmia could also be triggered by a myocardial injury and is not an uncommon occurrence in myocarditis or pericarditis [14,15]. Although no study has found an association between arrhythmia and COVID-19 vaccines, our findings suggest that arrhythmia following vaccination in our population is an AESI of concern. We also observed a small increased risk of venous thromboembolism after BNT162b2 and ChAdOx1 vaccination while the higher risk of thrombocytopenia was significant for ChAdOx1 vaccine. Although the elevated risk of these events indicates potential association with the vaccines, the low absolute number of events, especially within ChAdOx1 has to be noted and the significant association in the main and subgroup analyses comes with a broad confidence interval.

Our results are consistent with and extend the findings of prior studies on the risk of serious adverse events following COVID-19 vaccination [11,16,17]. Vaccine-induced immune thrombotic thrombocytopenia (VITT) is one of the rare, serious events reported

Table 1
Characteristics of events that occurred in 21 days after either dose of COVID-19 vaccine among vaccinated population in Malaysia from 1 February 2021 to 30 September 2021.

	Thrombo cytopenia	Venous thrombo embolism	Myocardial infarction	Myocarditis / pericarditis	Arrhythmia	Stroke, ischaemic	Stroke haemorrhagic	Bell's Palsy	Convulsion / seizure
Total event	206	307	1495	25	1375	1840	401	53	401
Dose 1	104 (50.5)	187 (60.9)	799 (53.4)	18 (72.0)	758 (55.1)	1005 (54.6)	236 (58.9)	33 (62.3)	218 (54.4)
Dose 2	102 (49.5)	120 (39.1)	696 (46.6)	7 (28.0)	617 (44.9)	835 (45.4)	165 (41.1)	20 (37.7)	183 (45.6)
Died	–	–	3 (0.2)	–	–	3 (0.2)	2 (0.5)	–	–
Vaccine platform									
BNT162b2	130 (63.1)	166 (54.1)	796 (53.2)	14 (56.0)	834 (60.7)	1006 (54.7)	199 (49.6)	27 (50.9)	227 (56.6)
CoronaVac	64 (31.1)	122 (39.7)	644 (43.1)	9 (36.0)	485 (35.3)	768 (41.7)	186 (46.4)	21 (39.6)	153 (38.2)
ChAdOx1	12 (5.8)	18 (5.9)	55 (3.7)	2 (8.0)	53 (3.9)	64 (3.5)	14 (3.5)	5 (9.4)	21 (5.2)
Others	–	1 (0.3)	–	–	3 (0.2)	2 (0.1)	2 (0.5)	–	–
Male	116 (56.3)	170 (55.4)	1121 (75.0)	15 (60.0)	731 (52.8)	1096 (59.6)	247 (61.6)	27 (50.9)	229 (57.1)
Age (years)									
Mean (SD)	57.5 (16.7)	56.6 (15.2)	60.3 (12.7)	43.6 (15.2)	62.8 (16.6)	63.6 (12.6)	59.9 (16.4)	50.1 (15.2)	50.6 (18.4)
18–39	40 (19.4)	52 (16.9)	97 (6.5)	11 (44.0)	176 (12.7)	75 (4.1)	48 (12.0)	15 (28.3)	142 (35.4)
40–59	63 (30.6)	110 (35.8)	589 (39.4)	10 (40.0)	287 (20.7)	577 (31.4)	130 (32.4)	20 (37.7)	115 (28.7)
60+	103 (50.0)	145 (47.2)	809 (54.1)	4 (16.0)	921 (66.5)	1188 (64.6)	222 (55.4)	18 (34.0)	144 (35.9)
Ethnicity									
Malay	128 (62.1)	208 (67.8)	887 (59.3)	11 (44.0)	814 (58.8)	1070 (58.2)	228 (56.9)	29 (54.7)	245 (61.1)
Chinese	33 (16.0)	40 (13.0)	258 (17.3)	5 (20.0)	350 (25.3)	452 (24.6)	100 (24.9)	16 (30.2)	70 (17.5)
Indian	16 (7.8)	20 (6.5)	186 (12.4)	4 (16.0)	69 (5.0)	140 (7.6)	16 (4.0)	5 (9.4)	40 (10.0)
Others	29 (14.1)	35 (11.4)	137 (9.2)	5 (20.0)	143 (10.3)	157 (8.5)	50 (12.5)	2 (3.8)	41 (10.2)
Non-Malaysian	–	3 (1.0)	27 (1.8)	–	7 (0.5)	18 (1.0)	7 (1.7)	1 (1.9)	4 (1.0)
Comorbidities									
Hypertension	90 (43.7)	129 (42.0)	759 (50.8)	4 (16.0)	735 (53.1)	1025 (55.7)	182 (45.4)	18 (34.0)	119 (29.7)
Diabetes	56 (27.2)	91 (29.6)	556 (37.2)	4 (16.0)	426 (30.8)	721 (39.2)	94 (23.4)	15 (28.3)	73 (18.2)
Heart disease	17 (8.3)	19 (6.2)	268 (17.9)	3 (12.0)	293 (21.2)	186 (10.1)	30 (7.5)	5 (9.4)	23 (5.7)
Dyslipidaemia	–	–	–	–	–	–	1 (0.2)	–	1 (0.2)

Data are presented as n (%) except otherwise stated. Abbreviation: SD, standard deviation. Numbers may not add up to the total due to the missing value.

after COVID-19 vaccination which was more common with ChAdOx1 than with other COVID-19 vaccines [18,19]. A recent study from England based on clinically validated cases from hospitals estimated the risk of thrombosis with thrombocytopenia syndrome after ChAdOx1 vaccination was higher in 18 to 39 years old than in the older population, highlighting the difference in risk between different age groups [20]. In our study, the absolute event rate for thrombosis and thrombocytopenia cases was lower than those observed in other countries [16,21,22]. Increased risk of myocarditis following receipt of mRNA COVID-19 vaccines has been described in several studies [23–25]. We did not observe the elevated risk of myocarditis/pericarditis for the population in Malaysia vaccinated with BNT162b2 during the study period and the number of events was too small for meaningful comparison by subgroup. Information to date indicates that these events occur more commonly in male, adolescent/young adults following mRNA vaccination [26]. The present analysis covers the period when vaccination for adolescents in Malaysia was just initiated (September 2021) and during this early period, there was no record of hospitalisation for the outcome events among the adolescent group. In terms of neurological complications, there were mixed findings on the association with COVID-19 vaccines. Previous study from the UK observed an increased risk of Bell's Palsy and Guillain-Barre syndrome following ChAdOx1 vaccination [27] while a study from Hong Kong reported risk of Bell's Palsy linked to CoronaVac vaccine [28]. Li et al. conducted a population-based study in the United Kingdom and Spain [29] and did not find safety signal for the risk of neurological events among those vaccinated with BNT162b2 and ChAdOx1 vaccine. Our findings are in line with the latter study, with no elevated risk of Bell's Palsy or Guillain-Barre syndrome seen for either BNT162b2, ChAdOx1, or CoronaVac. Compared to other vaccines, there was less information available on real-world data of AESIs among CoronaVac recipients. Our study further showed that the risk for most AESIs was found to be not significantly increased in those receiving CoronaVac. There was a slight elevation in the risk of myocardial infarction and ischaemic stroke after CoronaVac vaccination when the analysis was conducted by dose and sex-specific, which was not observed with other vaccines. Yet, this result must be interpreted cautiously since subgroup analyses lack power, and evidence on serious events with CoronaVac vaccination are still accruing for comparison on the magnitude of risk.

Although many cardiovascular and cerebrovascular events were reported following vaccination, a causality assessment needs to be conducted to confirm the associations because these events are prevalent among the population in Malaysia. Myocardial infarction and ischaemic stroke are the top leading cause of death in Malaysia for over a decade where on average, 90 to 100 hospital admissions

Table 2
Absolute rate of outcome events within 21 days of COVID-19 vaccination by vaccine type.

	BNT162b2	CoronaVac	ChAdOx1
Thrombocytopenia			
No. of events	130	64	12
Event rate per 1 million doses administered	8.45	3.76	4.37
Event rate per 1 million vaccinated persons	14.88	6.83	6.10
Venous thromboembolism			
No. of events	166	122	18
Event rate per 1 million doses administered	10.79	7.16	6.56
Event rate per 1 million vaccinated persons	19.00	13.02	9.14
Myocardial infarction			
No. of events	796	644	55
Event rate per 1 million doses administered	51.73	37.82	20.04
Event rate per 1 million vaccinated persons	91.12	68.73	27.94
Myocarditis/pericarditis			
No. of events	14	9	2
Event rate per 1 million doses administered	0.91	0.53	0.73
Event rate per 1 million vaccinated persons	1.60	0.96	1.02
Arrhythmia			
No. of events	834	485	53
Event rate per 1 million doses administered	54.20	28.48	19.31
Event rate per 1 million vaccinated persons	95.47	51.76	26.92
Stroke, ischaemic			
No. of events	1006	768	64
Event rate per 1 million doses administered	65.38	45.10	23.32
Event rate per 1 million vaccinated persons	115.16	81.96	32.51
Stroke, haemorrhagic			
No. of events	199	186	14
Event rate per 1 million doses administered	12.93	10.92	5.10
Event rate per 1 million vaccinated persons	22.78	19.85	7.11
Bell's Palsy			
No. of events	27	21	5
Event rate per 1 million doses administered	1.75	1.23	1.82
Event rate per 1 million vaccinated persons	3.09	2.24	2.54
Convulsion/seizure			
No. of events	227	153	21
Event rate per 1 million doses administered	14.75	8.98	7.65
Event rate per 1 million vaccinated persons	25.99	16.33	10.67

Denominator is total vaccine doses administered and total individuals vaccinated up to 31 August 2021.

occurred each day due to these events [30,31]. Despite including incidence cases in the analysis, the study cohort still comprised patients with other risk factors and comorbidities which could lead to the development of events regardless of vaccination status. The risk of events can also be attributed to the population selected for vaccination. The elderly and high-risk populations were prioritised for vaccination and some events such as cardiovascular complications are more commonly seen in these populations. Due to the opt-in policy for ChAdOx1 vaccination in Malaysia, this group include those of younger age categories. This is unlike other countries that limit the use of ChAdOx1 in the older age group amid safety concerns that are more prevalent in the younger population [32]. Therefore, the population vaccinated with ChAdOx1 is considered to be "healthier" than those who received other vaccines since individuals without health concerns are more likely to sign up for this cohort. Moreover, there were substantially fewer second doses of ChAdOx1 vaccines given during the study period due to the longer dose interval of 9–12 weeks for the administration of the second dose according to the policy and recommendation adopted in Malaysia [33].

Based on the distribution of event occurrence over time, our finding suggests that the 3-week time period following vaccination is a crucial period for monitoring AESI and any event that occurs during this period warrants further investigation on the potential association to vaccines. Nevertheless, events presented later should not be disregarded since both short- and long-term risks need to be assessed. In studies evaluating the association between AESI and COVID-19 vaccines, the period considered at risk ranged between 21 and 28 days after vaccination with at least 3 months duration for the overall surveillance period [16,17,22,34]. The length of time for monitoring AESI after vaccination should have a sufficient duration of follow-up to include events that occur at different time points relative to vaccine exposure to detect any important risk [5,35].

4.1. Strength and limitations

To the best of our knowledge, this is one of the few large population-based studies that provide evidence of risk estimate for serious adverse events after vaccination in the population between the three different vaccine platforms. Our study addresses the limitation of data capture via a passive surveillance system by including a longer follow-up period to monitor outcome occurrence among vaccinated populations and identify the potential association with the vaccine. Furthermore, ascertainment of hospitalisations and vaccination status was conducted independently which allow us to capture more events and we utilised the SCCS design to provide estimates of risk on both relative scale (incidence rate ratios) and absolute scale (rate per million vaccinated persons). The SCCS design is an established method in vaccine safety study that uses within-person comparison to control all constant confounding factors during the study period. We also adjusted for the calendar period to account for potential temporal confounding. In this study, we provide additional context on a range of outcomes among CoronaVac vaccinees, which has not been frequently reported in large observational studies so far.

There are several limitations in this study that we acknowledge. We used secondary databases of the vaccinated and hospitalised; hence we cannot completely rule out misclassification bias and unmeasured confounding. We used hospitalisations as the study outcomes which captures events of a more serious nature that are admitted to hospitals for diagnosis or treatment. It is possible that some people who have had vaccination and developed the event were not hospitalised or died before being admitted. Ascertainment of outcomes was based on diagnosis codes, which might overestimate the risk without including other parameters to define the case; for instance, diagnosis of VITT and myocarditis are usually accompanied by laboratory parameters. Lastly, some of the predefined outcome events are rare, resulting in limited statistical power and wide confidence interval. Larger study cohorts and additional data over extended period of time will be useful for more detailed analysis.

4.2. Policy implications

Compared with the total number of vaccine doses given, our findings show that the incidence of all serious events are relatively low. There was an overall increased risk of certain events within the 21 days after vaccination compared to the control period, but

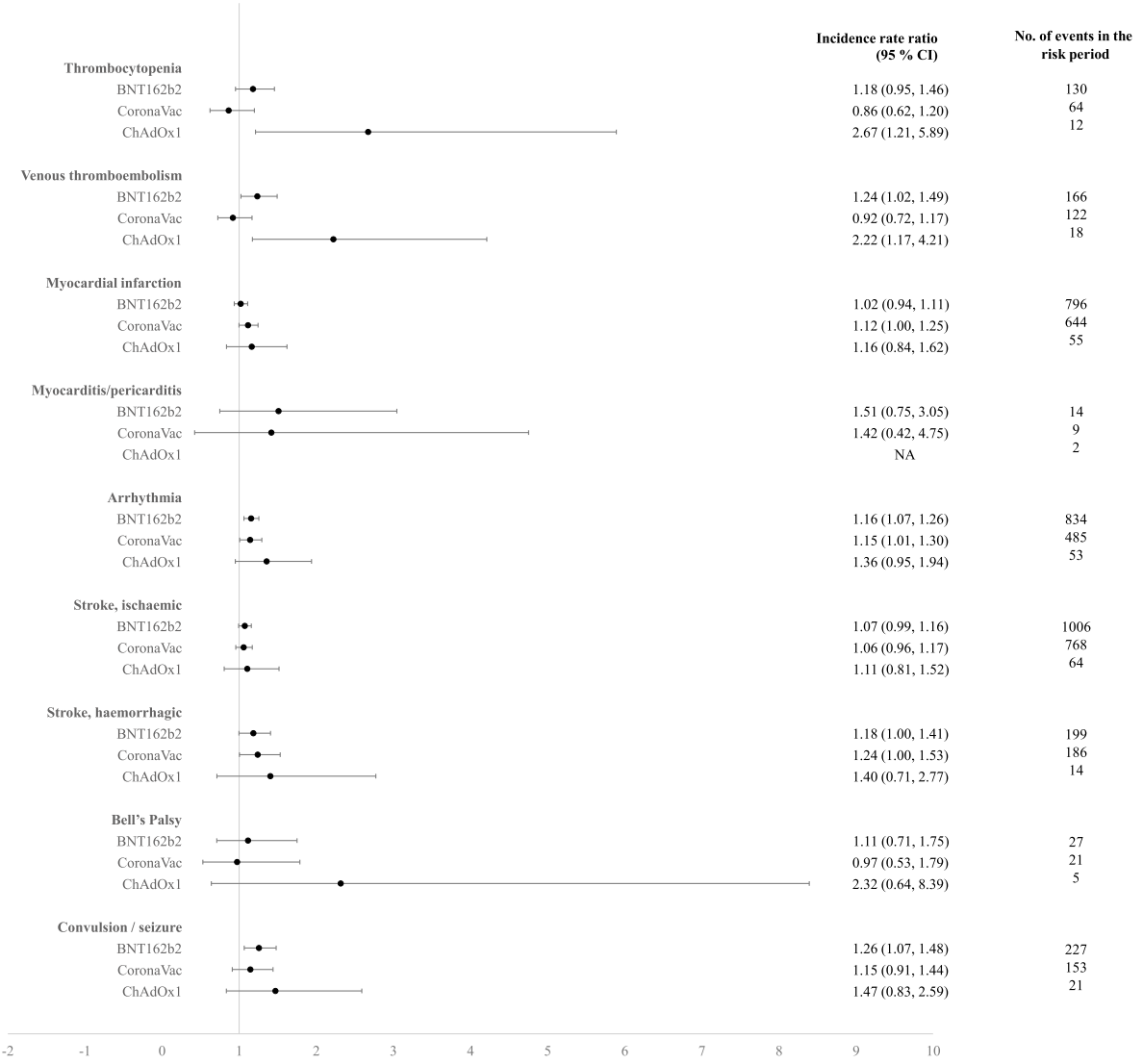


Fig. 2. Incidence rate ratios and 95% confidence intervals of outcome events in the 21-day risk period after either dose of BNT162b2, CoronaVac, and ChAdOx1 vaccines compared with outcome events in the control period, adjusted for calendar month between 1 February 2021 to 30 September 2021. Abbreviation: NA, not available.

the weak association requires further consideration of the clinical importance of the results. Given that COVID-19 remain prevalent and the vaccines provide nearly 90% protection against infection, the benefits of vaccination still far outweigh the risks [36]. Furthermore, studies have demonstrated that the risk of serious events associated with the COVID-19 infection itself was higher than the risk from vaccination [16,23,27]. Although the estimated risks of these serious adverse events in our population might be higher or lower than those reported in other countries, these initial findings provide valuable information that could help to inform clinical decision making and policymakers, especially in the risk-benefit assessments and subsequent immunisation plan.

5. Conclusion

Overall, the study demonstrates the safety of COVID-19 vaccines concerning the study outcomes among recipients of BNT162b2, CoronaVac, and ChAdOx1 vaccines. Our findings suggest that serious events of concern within our population include thrombocytopenia, venous thromboembolism, convulsion/seizure, and arrhythmia, although the numbers were relatively small and further exploration of the causal link with vaccination is warranted. More importantly, the overall benefits of COVID-19 vaccines outweigh the potential risks and this information needs to be interpreted in conjunction with vaccine effectiveness data for risk-benefit analysis of the vaccination program. Ongoing monitoring of COVID-19 vaccine safety is required and the information will be regularly updated as more data accumulate to assess short- and long-term complications with vaccination.

Funding

This research was supported by a grant from the Ministry of Health Malaysia – Sukuk Prihatin (NMRR-21-822-59745). The funding body had no role in the study design, data analysis, or the presentation of data and writing of the manuscript.

Table 3
Incidence rate ratios of outcome events in the 21-day risk period after COVID-19 vaccination by vaccine type and dose number, adjusted for calendar month between 1 February 2021 to 30 September 2021.

	Dose 1		Dose 2	
	No. of events	Incidence rate ratio (95% CI)	No. of events	Incidence rate ratio (95% CI)
Thrombocytopenia				
BNT162b2	66	1.09 (0.83, 1.43)	64	1.29 (0.98, 1.70)
CoronaVac	28	0.68 (0.44, 1.05)	36	1.09 (0.73, 1.62)
ChAdOx1	10	2.58 (1.13, 5.90)	2	3.44 (0.64, 18.6)
Venous thromboembolism				
BNT162b2	103	1.34 (1.07, 1.67)	63	1.09 (0.83, 1.44)
CoronaVac	68	0.94 (0.71, 1.26)	54	0.89 (0.65, 1.22)
ChAdOx1	15	2.52 (1.30, 4.92)	3	1.24 (0.33, 4.66)
Myocardial infarction				
BNT162b2	409	0.97 (0.87, 1.08)	387	1.08 (0.97, 1.21)
CoronaVac	356	1.16 (1.02, 1.32)	288	1.06 (0.92, 1.23)
ChAdOx1	34	1.02 (0.69, 1.51)	21	1.58 (0.93, 2.67)
Myocarditis/pericarditis				
BNT162b2	9	1.52 (0.67, 3.46)	5	1.13 (0.41, 3.09)
CoronaVac	7	1.97 (0.57, 6.74)	2	0.67 (0.12, 3.83)
ChAdOx1	2	**	–	–
Arrhythmia				
BNT162b2	437	1.12 (1.01, 1.25)	397	1.19 (1.07, 1.33)
CoronaVac	276	1.18 (1.02, 1.37)	209	1.10 (0.93, 1.29)
ChAdOx1	42	1.51 (1.03, 2.23)	11	0.96 (0.49, 1.90)
Stroke, ischaemic				
BNT162b2	535	1.05 (0.95, 1.15)	471	1.11 (1.00, 1.23)
CoronaVac	421	1.05 (0.93, 1.18)	347	1.07 (0.94, 1.22)
ChAdOx1	47	1.14 (0.80, 1.63)	17	1.01 (0.58, 1.76)
Stroke, haemorrhagic				
BNT162b2	119	1.29 (1.05, 1.59)	80	1.05 (0.82, 1.34)
CoronaVac	105	1.31 (1.02, 1.68)	81	1.16 (0.89, 1.52)
ChAdOx1	11	1.32 (0.63, 2.79)	3	1.80 (0.47, 6.94)
Bell's Palsy				
BNT162b2	17	1.32 (0.77, 2.24)	10	0.88 (0.45, 1.73)
CoronaVac	12	1.06 (0.52, 2.18)	9	0.87 (0.40, 1.92)
ChAdOx1	4	2.88 (0.74, 11.2)	1	1.09 (0.10, 12.4)
Convulsion/seizure				
BNT162b2	116	1.15 (0.94, 1.42)	111	1.39 (1.12, 1.72)
CoronaVac	83	1.12 (0.86, 1.47)	70	1.17 (0.87, 1.57)
ChAdOx1	19	1.55 (0.88, 2.75)	2	0.85 (0.18, 3.94)

** Values are suppressed for total event <5. Rate ratio estimates for comparison with control period of day 22 after the last vaccine dose until 30 September 2021 (post vaccination control) and from 1 February 2021 until 15 days before vaccination (pre-vaccination control). Day of vaccination (day 0) and 14 days before vaccination (pre-risk period) were included as separate risk periods. Abbreviations: CI, confidence interval; n, number of events in the risk period.

The SAFECOVAC study group

Members of the SAFECOVAC study group are listed in Supplement 8.

CRediT authorship contribution statement

Norazida Ab Rahman: Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization, Funding acquisition. **Ming Tsuey Lim:** Investigation, Writing – review & editing, Project administration. **Fei Yee Lee:** Investigation, Writing – review & editing, Project administration. **Sing Chet Lee:** Investigation, Writing – review & editing. **Azuana Ramli:** Investigation, Writing – review & editing. **Siti Nurhafizah Saharudin:** Investigation, Writing – review & editing. **Teck Long King:** Investigation, Writing – review & editing. **Emelyne Bani Anak Jam:** Investigation, Writing – review & editing. **Nor Aliya Ayub:** Investigation, Writing – review & editing. **Raj Kumar Sevalingam:** Investigation, Writing – review & editing. **Rashidah Bahari:** Investigation, Writing – review & editing. **Nor Nadziroh Ibrahim:** Investigation, Writing – review & editing. **Fatihah Mahmud:** Investigation, Writing – review & editing. **Sheamini Sivasampu:** . **Kalaiarasu M Peariasamy:** Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the Director General of Health Malaysia for his permission to publish this study. We also thank all the investigators and collaborators for their immense contribution and support. We would like to acknowledge all data providers who make data available for research and research assistants who were involved in data collection.

Data Availability

The data that supports the findings of this study are available within the article and its supplementary materials. Access to datasets is provided by the corresponding data custodians for analysis for this study, but we have no permission to make generated datasets available. Malaysia COVID-19 vaccine administration data are available at https://github.com/MoH-Malaysia/covid19-public, redacted of personal identifying information.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.05.075.

References

[1] Ritchie H, Mathieu E, Rodés-Guirao L, Appel C, Giattino C, Ortiz-Ospina E, et al. Coronavirus (COVID-19) Vaccinations Published online at OurWorldInData.org; 2020 [Available from: https://ourworldindata.org/covid-vaccinations.
[2] Chandler RE. Optimizing safety surveillance for COVID-19 vaccines. Nat Rev Immunol 2020;20(8):451–2.
[3] Griffin MR, Braun MM, Bart KJ. What should an ideal vaccine postlicensure safety system be? Am J Public Health 2009;99(S2):S345–50.
[4] Lei J, Balakrishnan MR, Gidudu JF, Zuber PLF. Use of a new global indicator for vaccine safety surveillance and trends in adverse events following immunization reporting 2000–2015. Vaccine 2018;36(12):1577–82.
[5] COVID-19 vaccines: safety surveillance manual, second edition. Geneva: World Health Organization; 2021.
[6] Mallapaty S. China's COVID vaccines have been crucial - now immunity is waning. Nature 2021;598(7881):398–9.
[7] COVID-19 Immunisation Task Force Malaysia (CITF-Malaysia) Ministry of Health Malaysia. Open data on Malaysia's National COVID-19 Immunisation Programme. 2021 [Available from: https://github.com/citf-malaysia/citf-public.
[8] Malaysian Pharmacovigilance Guidelines (Second Edition). Selangor, Malaysia: National Pharmaceutical Regulatory Agency (NPRA), Ministry of Health Malaysia; 2016.
[9] Malaysian Health Data Warehouse (MyHDW) - 2015-2016 Start up: initiation. Malaysia: Health Informatics Centre, Planning Division, Ministry of Health Malaysia; 2017.
[10] Weldeselassie YG, Whitaker HJ, Farrington CP. Use of the self-controlled case-series method in vaccine safety studies: review and recommendations for best practice. Epidemiol Infect 2011;139(12):1805–17.
[11] Jabagi MJ, Botton J, Bertrand M, Weill A, Farrington P, Zureik M, et al. Myocardial Infarction, Stroke, and Pulmonary Embolism After BNT162b2 mRNA COVID-19 Vaccine in People Aged 75 Years or Older. JAMA 2022;327(1):80. https://doi.org/10.1001/jama.2021.21699.
[12] Quer G, Gadaleta M, Radin JM, Andersen KG, Baca-Motes K, Ramos E, et al. The Physiologic Response to COVID-19 Vaccination. medRxiv : the preprint server for health sciences, 2021.
[13] Taylor S, Asmundson GJG. Immunization stress-related responses: Implications for vaccination hesitancy and vaccination processes during the COVID-19 pandemic. J Anxiety Disord 2021;84:102489. https://doi.org/10.1016/j.janxdis.2021.102489.
[14] Shiravi AA, Ardekani A, Sheikhbahaei E, Heshmat-Ghahdarijani K. Cardiovascular Complications of SARS-CoV-2 Vaccines: An Overview. Cardiol. Therapy 2022;11(1):13–21.
[15] Scheuermeyer FX, Yoo J, Greene M, O'Donnell S. Atrial fibrillation as a precursor of mRNA-1273 SARS-CoV-2 vaccine-induced pericarditis. Can J Emerg Med 2022;24(2):230–2.
[16] Hippisley-Cox J, Patone M, Mei XW, Saatci D, Dixon S, Khunti K, et al. Risk of thrombocytopenia and thromboembolism after covid-19 vaccination and SARS-CoV-2 positive testing: self-controlled case series study. BMJ (Clinical research ed). 2021;374:n1931.
[17] Klein NP, Lewis N, Goddard K, Fireman B, Zerbo O, Hanson KE, et al. Surveillance for Adverse Events After COVID-19 mRNA Vaccination. JAMA 2021;326(14):1390. https://doi.org/10.1001/jama.2021.15072.
[18] Pavord S, Scully M, Hunt BJ, Lester W, Bagot C, Craven B, et al. Clinical Features of Vaccine-Induced Immune Thrombocytopenia and Thrombosis. N Engl J Med 2021;385(18):1680–9.
[19] Al-Ali D, Elshafeey A, Mushannen M, Kawas H, Shafiq A, Mhaimeed N, et al. Cardiovascular and haematological events post COVID-19 vaccination: A systematic review. J Cell Mol Med 2022;26(3):636–53.
[20] Higgins H, Andrews N, Stowe J, Amirthalingam G, Ramsay M, Bahra G, et al. Risk of thrombosis with thrombocytopenia syndrome after COVID-19 vaccination prior to the recognition of vaccine-induced thrombocytopenia and thrombosis: A self-controlled case series study in England. Res Pract Thrombosis Haemostasis 2022;6(3). https://doi.org/10.1002/rth2.v6.310.1002/rth2.12698.
[21] Simpson CR, Shi T, Vasileiou E, Katikireddi SV, Kerr S, Moore E, et al. First-dose ChAdOx1 and BNT162b2 COVID-19 vaccines and thrombocytopenic, thromboembolic and hemorrhagic events in Scotland. Nat Med 2021;27(7):1290–7.
[22] Whiteley WN, Ip S, Cooper JA, Bolton T, Keene S, Walker V, et al. Association of COVID-19 vaccines ChAdOx1 and BNT162b2 with major venous, arterial, or thrombocytopenic events: A population-based cohort study of 46 million adults in England. PLoS Med 2022;19(2):e1003926. https://doi.org/10.1371/journal.pmed.1003926.
[23] Patone M, Mei XW, Handunnetthi L, Dixon S, Zaccardi F, Shankar-Hari M, et al. Risks of myocarditis, pericarditis, and cardiac arrhythmias associated with COVID-19 vaccination or SARS-CoV-2 infection. Nat Med 2022;28(2):410–22.
[24] Witberg G, Barda N, Hoss S, Richter I, Wiessman M, Aviv Y, et al. Myocarditis after Covid-19 Vaccination in a Large Health Care Organization. New Engl. J Med 2021;385(23):2132–9.
[25] Bozkurt B, Kamat I, Hotez PJ. Myocarditis With COVID-19 mRNA Vaccines. Circulation 2021;144(6):471–84.
[26] Centers for Disease Control and Prevention National Center for Immunization & Respiratory Diseases. COVID-19 vaccine safety update: Advisory Committee on Immunization Practices (ACIP) [Webinar] Atlanta, GA: Centers for Disease Control and Prevention; 2021 [presented 23 Jun 2021; cited 20 Dec 2021]. Available from: https://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2021-06/03-COVIDShimabukuro-508.pdf.
[27] Patone M, Handunnetthi L, Saatci D, Pan J, Katikireddi SV, Razvi S, et al. Neurological complications after first dose of COVID-19 vaccines and SARS-CoV-2 infection. Nat Med 2021;27(12):2144–53.
[28] Wan EYF, Chui CSL, Lai FTT, Chan EWY, Li X, Yan VKC, et al. Bell's palsy following vaccination with mRNA (BNT162b2) and inactivated (CoronaVac) SARS-CoV-2 vaccines: a case series and nested case-control study. Lancet Infect Dis 2022;22(1):64–72.
[29] Li X, Raventós B, Roel E, Pistillo A, Martinez-Hernandez E, Delmestri A, et al. Association between covid-19 vaccination, SARS-CoV-2 infection, and risk of immune mediated neurological events: population based cohort and self-controlled case series analysis. BMJ (Clinical research ed). 2022;376:e068373.
[30] Ministry of Health Malaysia. Health Facts 2019. 2019.
[31] Hwong WY, Ang SH, Bots ML, Sivasampu S, Selvarajah S, Law WC, et al. Trends of Stroke Incidence and 28-Day All-Cause Mortality after a Stroke in Malaysia: A Linkage of National Data Sources. Global Heart 2021;16(1). https://doi.org/10.5334/gh.79110.5334/gh.791.s1.
[32] Forman R, Jit M, Mossialos E. Divergent vaccination policies could fuel mistrust and hesitancy. Lancet (London, England) 2021;397(10292):2333. https://doi.org/10.1016/S0140-6736(21)01106-5.
[33] Ministry of Health Malaysia. Clinical guidelines on COVID-19 vaccination in Malaysia (4th Edition). Malaysia; 2021.
[34] Husby A, Hansen JV, Fosbøl E, Thiesson EM, Madsen M, Thomsen RW, et al. SARS-CoV-2 vaccination and myocarditis or myopericarditis: population based cohort study. BMJ (Clinical research ed). 2021;375:e068665.
[35] Kochhar S, Excler J-L, Bok K, Gurwith M, McNeil MM, Seligman SJ, et al. Defining the interval for monitoring potential adverse events following immunization (AEFIs) after receipt of live viral vectored vaccines. Vaccine 2019;37(38):5796–802.
[36] Suah JL, Tok PSK, Ong SM, Husin M, Tng BH, Sivasampu S, et al. PICK-ing Malaysia's Epidemic Apart: Effectiveness of a Diverse COVID-19 Vaccine Portfolio. Vaccines 2021;9(12):1381. https://doi.org/10.3390/vaccines9121381.

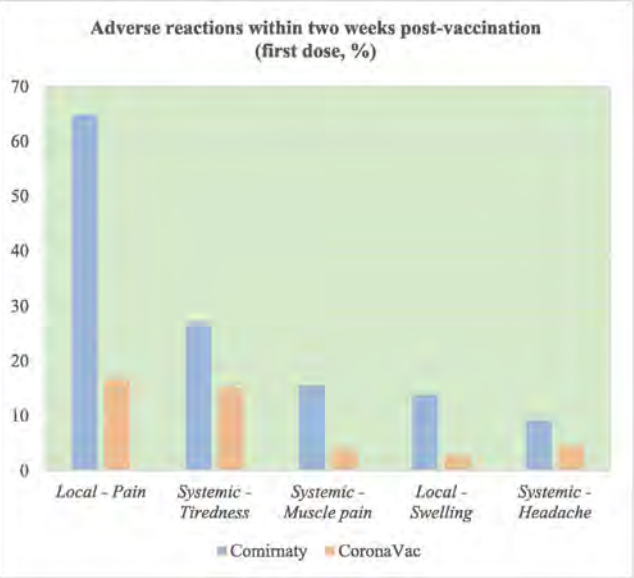
8.3. CoronaVac tem 83% menos chance de causar efeitos adversos do que vacinas de RNA mensageiro

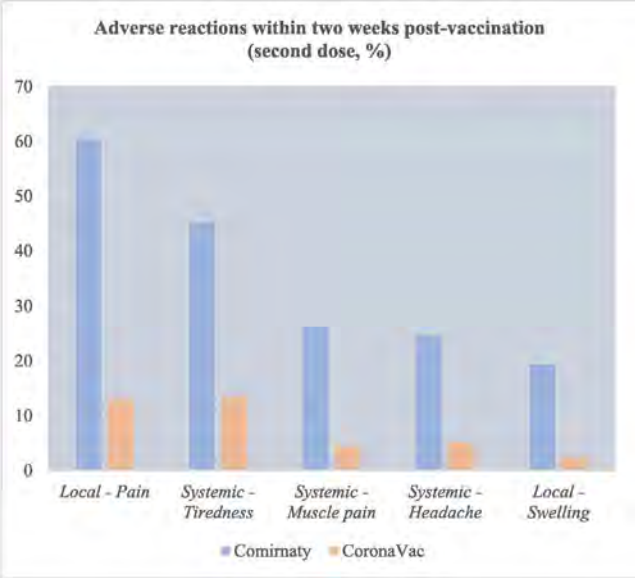
Um estudo publicado na revista Vaccines mostrou que quem toma a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, tem 83% menos chance de ter reações adversas do que pessoas que recebem vacinas de RNA mensageiro (mRNA). O trabalho foi conduzido entre fevereiro e julho de 2021 por pesquisadores do Departamento de Saúde de Hong Kong, da Universidade de Hong Kong, do Parque de Ciência e Tecnologia de Hong Kong e da Universidade de Londres.

"A análise ajustada sugere que, em comparação com a Comirnaty [nome oficial da vacina da Pfizer], a CoronaVac está associada a 83% menos chance de causar qualquer reação adversa e 76% menos de chance de reações adversas sistêmicas", descreveu o estudo.

Os cientistas recrutaram 1.129 indivíduos que receberam a CoronaVac, com idade média de 46 anos, e 969 pessoas que tomaram a vacina de mRNA da Pfizer, com idade média de 43 anos. Os voluntários foram acompanhados durante 14 dias após cada dose, período no qual responderam a um questionário sobre reações adversas causadas pela vacinação.

Durante o acompanhamento, 82,7% dos participantes que tomaram o imunizante da Pfizer reportaram eventos adversos, enquanto 48,1% dos vacinados com a CoronaVac relataram alguma reação. Os sintomas mais comuns após primeira e segunda dose para ambos os imunizantes foram dor e inchaço no local da injeção, fadiga, dor muscular e dor de cabeça. O gráfico compara





a porcentagem de reações entre as duas vacinas.

Segurança da CoronaVac já foi comprovada por outros estudos

A pesquisa corrobora os achados de outros trabalhos já publicados, como ensaios clínicos da Turquia e da China, que demonstraram que a CoronaVac pode causar eventos adversos em apenas 18,9% a 33% dos indivíduos, apresentando um alto perfil de segurança.

Enquanto estudo clínico de fase 3 da vacina Comirnaty demostrou que aproximadamente 80% dos voluntários apresentaram reações adversas após terem tomado o imunizante.

Segundo os cientistas, estudos já mostraram que a reatogenicidade é um dos fatores que influenciam a decisão da população sobre se vacinar ou não. Pesquisas que esclareçam os possíveis efeitos adversos e atestem a segurança das vacinas são importantes para aumentar a confiança nos imunizantes.

Publicado em: 7/02/2022

Vaccine xxx (xxxx) xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Self-reported reactogenicity of CoronaVac (Sinovac) compared with Comirnaty (Pfizer-BioNTech): A prospective cohort study with intensive monitoring

Francisco Tsz Tsun Lai [a,b], Miriam Tim Yin Leung [a,b], Edward Wai Wa Chan [a,b], Lei Huang [a,b], Lauren Ka Wun Lau [a,b], Kuan Peng [a,c], Janice Ching Nam Leung [a,b], Min Fan [a,b], Kailin Chen [a,b], Dawn Hei Lum [a], Xue Li [a,b,c], Celine Sze Ling Chui [b,d,e], Eric Yuk Fai Wan [a,b,f], Carlos King Ho Wong [a,b,f], Edwin Fung Shing Lam [g], Terence Yung Yan Cheung [g], Benjamin John Cowling [b,e], Ian Chi Kei Wong [a,b,h], Esther Wai Yin Chan [a,b,*]

[a] Centre for Safe Medication Practice and Research, Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China
[b] Laboratory of Data Discovery for. Health ($D^2$4H), Hong Kong Science and Technology Park, Hong Kong, China
[c] Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China
[d] School of Nursing, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China
[e] School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China
[f] Department of Family Medicine and Primary Care, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China
[g] Department of Health, Hong Kong Special Administration Region Government, Hong Kong, China
[h] Research Department of Practice and Policy, School of Pharmacy, University College London, London, United Kingdom

ARTICLE INFO

Article history:
Received 11 November 2021
Received in revised form 19 January 2022
Accepted 31 January 2022
Available online xxxx

Keywords:
Community vaccination center
Covid-19
Multimorbidity
Pain
SARS-CoV-2
Severe allergic reactions
Systemic reactions

ABSTRACT

Objective: CoronaVac (Sinovac) Covid-19 vaccine has recently been approved for emergency use by the World Health Organization. However, data on its reactogenicity in real-world settings is scant. This study aimed to compare self-reported post-vaccination adverse reactions between CoronaVac and Comirnaty (Pfizer-BioNTech).

Methods: We adopted a prospective cohort study design using online surveys from the day of first-dose vaccination with intensive follow-up through two weeks after the second dose (11 time points). The primary outcome was adverse reactions (any versus none) and secondary outcomes were the sub-categories of adverse reactions (local, systemic, and severe allergic reactions). Potential effect modification across multimorbidity status, older age, and sex was examined.

Results: In total, 2,098 participants who were scheduled to complete the 14th-day survey were included, with 46.2% receiving Comirnaty. Retention rate two weeks after the second dose was 81.0% for the CoronaVac group and 83.6% for the Comirnaty group. Throughout the follow-up period, 801 (82.7%) of those receiving Comirnaty and 543 (48.1%) of those receiving CoronaVac reported adverse reactions. Adjusted analysis suggested that compared with Comirnaty, CoronaVac was associated with 83%-reduced odds of any adverse reactions [adjusted odds ratio (AOR) = 0.17, 95% confidence interval (CI) 0.15–0.20], 92%-reduced odds of local adverse reactions (AOR = 0.08, 95% CI 0.06–0.09), and 76%-reduced odds of systemic adverse reactions (AOR = 0.24, 95% CI 0.16–0.28). No significant effect modification was identified.

Conclusion: This post-marketing study comparing the reactogenicity of Covid-19 vaccines suggests a lower risk of self-reported adverse reactions following vaccination with CoronaVac compared with Comirnaty.

1. Introduction

CoronaVac (Sinovac) Covid-19 vaccine, an inactivated virus vaccine, has been approved for emergency use by the World Health

* Corresponding author at: 1/F, Jockey Club Building for Interdisciplinary Research, 5 Sassoon Road, Pokfulam, Hong Kong, China.
E-mail address: ewchan@hku.hk (E.W.Y. Chan).

https://doi.org/10.1016/j.vaccine.2022.01.062
0264-410X/

Organization (WHO) [1]. Phase I/II [2] and phase III clinical trials [3] as well as preliminary post-marketing research [4] have presented reassuring data on the safety profile, indicated by the absence or rare incidence of adverse events of interest, and a satisfactory level of efficacy in the protection against Covid-19. Nevertheless, little research has examined its reactogenicity, i.e. a vaccine property with regard to the production of expected adverse reactions, particularly through active self-report data collection about typically mild to moderate and self-limiting reactions requiring minimal to no medical interventions [5]. The occurrence of adverse reactions is not directly correlated to efficacy level. No research has compared CoronaVac's reactogenicity with messenger RNA (mRNA) vaccines [6], which are developed on a different technological platform and typically more widely used in Western countries [7]. A prolonged absence of this important information may worsen the problem of vaccine hesitancy [8] and hamper our efforts in the fight against the pandemic.

Comirnaty (Pfizer-BioNTech) Covid-19 vaccine utilises mRNA for immunization against Covid-19 [9,10] As of July 2021, >100 countries have approved it for emergency use and rolled out massive vaccination programs. From published clinical data [11,12], it is observed that a relatively high proportion of vaccinated individuals reported discomfort or adverse reactions following vaccination [10,13]. In a large randomized controlled trial [10], approximately 80% of vaccinated adults aged 16–55 reported at post-vaccination adverse reactions following both doses (first dose: 83%; second dose: 78%) such as pain at the injection site, fatigue, dizziness, etc. This proportion is seemingly lower among those who received CoronaVac in clinical trials conducted in Turkey [14] and China [2], in which only 18.9 to 35.0% of vaccinated individuals reported adverse reactions within 28 days post-vaccination (second dose). The phase III clinical trial of BBIBP-CorV, another inactivated virus vaccine, also showed that only less than half of the vaccinated individuals had any adverse reactions (both doses combined) [15]. To our knowledge, the comparative reactogenicity of CoronaVac and Comirnaty is yet to be explored in the same population.

Hong Kong is among jurisdictions that has approved the emergency use of both vaccines and implemented publicly funded mass vaccination programs for residents' immunization against Covid-19 since February 2021 [16]. This study aims to describe and compare post-marketing, self-reported reactogenicity of CoronaVac and Comirnaty after both the first and second doses in this predominantly Chinese population, which represents highly important information especially in countries where the infection rate is low and the side effects of vaccines are of public concern. We hypothesized a milder reactogenicity of CoronaVac compared with Comirnaty. Potential effect modification of age, sex, and multimorbidity status on this difference was also examined.

2. Methods

2.1. Study design

Under the Covid-19 vaccines adverse events response and evaluation programme commissioned by the Hong Kong Government, we adopted a prospective cohort design with self-reported data collected on the first-dose vaccination day, as well as the first, second, third, seventh, and the fourteenth day following both doses of vaccination (11 time points). A 14-day follow-up period is consistent with the common existing literature and enhances the comparability of this research [12]. Baseline demographic and health status information were collected on the day of the first-dose and self-reports of adverse reactions of various types were collected throughout the observation period, i.e. all time points.

2.2. Participants

We recruited participants aged 16 or above receiving the first dose of either CoronaVac and Comirnaty at community vaccination centers run by the Government or at private clinics (only for CoronaVac) starting from 27th February 2021. We supplemented the active in-person recruitment with flyers including a quick-response (QR) link to the online survey distributed at healthcare facilities. The link to follow-up surveys was sent to participants via instant text messages and surveys were conducted online using Qualtrics, an online data collection platform. Only those participants who were scheduled to complete the 14th-day follow-up survey for the second dose according to the recommended dosing interval, i.e. number of days, between the two doses were included in the analysis. Participants could withdraw from the study anytime.

This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW-21–090) and the Department of Health Ethics Committee (LM 21/2021). Upon recruitment, written informed consent from the participants were obtained. The consent form, patient information leaflet, paper questionnaires can be downloaded from our website (https://www.hkcare.hku.hk/).

2.3. Outcomes

The primary outcome of this study was self-reported adverse reactions (any versus none). Secondary outcomes were dichotomous indicators of the three sub-categories of self-reported adverse reactions, including local (numbness, soreness, pain, swelling, redness, and itch), systemic (sore throat, tiredness, fever, chills, sweating, cough, headache, muscle pain, joint pain, pain in limbs, abdominal pain, diarrhea, nausea, vomiting, poor appetite, insomnia, feeling unwell, enlarged lymph nodes, rash, and temporary one-sided facial drooping), and severe allergic reactions (hypotension, dizziness, itchy skin rash, swelling of face or tongue, and wheezing/shortness of breath).

2.4. Exposure

Vaccine type (CoronaVac versus Comirnaty) was the primary exposure of the analysis because they were the only available vaccine options in Hong Kong. As a secondary exposure, we also compared the second dose of vaccination against the first dose.

2.5. Effect modifier

Multimorbidity, defined as the presence of two or more listed chronic conditions [17] (ankylosing spondylitis, asthma, psoriasis, rheumatoid arthritis, systemic lupus erythematosus, cancer remission, cancer under treatment, hypertension, hypercholesterolemia, heart disease, diabetes, stroke, neurological disorders, mental health disorders, liver problems, and kidney problems), was examined as an effect modifier in the association of vaccine type and adverse reactions. This list considered the prevalence and relevance of the conditions as well as the comparability of the findings with the existing literature [18]. We also examined sex (men versus women) and older age (60 or more versus 59 or less) as potential effect modifiers.

2.6. Multivariable adjustment

At the person-level, covariates including age, sex (men versus women), educational attainment (primary or below, secondary, post-secondary, and university or above), history of allergy to medications and to food (any versus none), smoking status (non-

smoker, former smoker, and current smoker), alcohol use (non-drinker, former drinker, occasional drinker, and regular drinker), number of chronic medications (none, 1–2, 3–4, 5–9, and 10 or more), and a range of chronic conditions (binary indicators, as listed above) were included for multivariable adjustment.

At the measurement level (each follow-up survey), specific follow-up days (vaccination day, first-, second-, third-, seventh-, and fourteenth-day post-vaccination) and second-dose (versus the first) were also adjusted for.

2.7. Statistical analysis

A random-intercept logistic regression model was implemented to examine the association between vaccine type (CoronaVac versus Comirnaty) and adverse reactions with multivariable adjustment where only the intercept was specified as random and the other factors as fixed. Individual participants were treated as a random factor. Listwise deletion was applied for missing data. We conducted sensitivity analyses with one-to-one propensity score matching (nearest-neighbor approach, caliper = 0.01) and inverse probability of treatment weighting based on the same person-level covariates respectively, was used as alternative approaches to multivariable adjustment to test the robustness of the results. We investigated the potential effect modification on this association by testing for the interaction between potential modifiers and vaccine type in extended models.

Stratified by vaccine type, a secondary analysis was conducted to compare the first and second dose of vaccination in terms of the association with adverse reactions. In the analyses, it was assumed that the assumption for the model, normal distribution of the random intercept, was true.

2.8. Sample size consideration

According to the widely adopted events-per-variable rule of thumb of 50 [19], we estimated we required 1,500 participants for a list of 30 covariates. We took a prudent approach and recruited over one-third more than this number to maximize the power of this study.

3. Results

As of 5th July 2021, 1,129 participants receiving CoronaVac and 969 receiving Comirnaty were recruited and were scheduled to complete the 14th-day follow-up survey for the second dose. For the 14th-day follow-up survey following the second dose, the retention rate was 81.0% for the CoronaVac group and 83.6% for the Comirnaty group. Response rates by follow-up day and vaccine type are tabulated as **eTable 1**. Chi-square tests showed that for Day 2, 3, and 7 for both doses, the Comirnaty group had a higher response rate ($P < 0.05$) although both groups had response rates exceeding 80% throughout the follow-up period.

3.1. Cohort characteristics

As shown in Table 1, the 46.7% of the CoronaVac group and 51.7% of the Comirnaty group were men. Mean age was 46.5 years for CoronaVac compared with 43.1 for Comirnaty. In total, 49.6% (CoronaVac) and 63.0% of the participants attained university education level. Current smokers constituted 10.1% (CoronaVac) and 5.9% (Comirnaty) of the groups, and 8.3% (CoronaVac) and 11.5% (Comirnaty) were regular drinkers. Around one-fifth of the participants were on at least one chronic medication at the time of vaccination for both vaccine groups. There were 7.3% (CoronaVac) and 5.8% (Comirnaty) of the participants who had a history of allergy to medications and 6.2% (CoronaVac) and 6.7% (Comirnaty) to food and other substances. For both groups, hypertension was the most prevalent chronic condition among participants (9.0 % for CoronaVac; 10.3% for Comirnaty), followed by hypercholesterolemia (7.2% for CoronaVac; 7.6% for Comirnaty) and diabetes (2.8% for CoronaVac; 3.6% for Comirnaty).

3.2. Adverse reactions

Throughout the follow-up period, 801 (82.7%) of those receiving Comirnaty and 543 (48.1%) of those receiving CoronaVac reported adverse reactions of any type. Among those reporting any adverse reactions at any time point following the first dose (n = 1,082), 65.6% reported adverse reactions at some point following the second, but among those who did not have adverse reactions at any time point following the first dose (n = 1,016), only 25.8% reported adverse reactions at some point following the second dose.

Fig. 1 shows the proportion [with 95% confidence interval (CI)] of participants reporting any type of adverse reactions at each time point throughout the observation period. For both vaccines, this proportion peaked on the first day post-vaccination and gradually declined. In general, more participants reported adverse reactions following the second rather than the first dose. **eFigure 1**, **eFigure 2** and **eFigure 3** show the proportion of participants reporting local, systemic, and severe allergic reactions throughout the follow-up period respectively, with largely similar patterns observed.

Fig. 2 are bar charts showing the five most commonly reported adverse reactions by vaccine type and dose (first versus second) two weeks post-vaccination. For both doses, pain at injection site, tiredness, muscle pain, headache, and swelling at the injection site were the five most frequently reported adverse reactions.

3.3. Multivariable adjusted analysis

As shown in Table 2, our random-intercept logistic regression model suggested that compared with Comirnaty, receiving CoronaVac was associated with 83%-reduced odds of any adverse reactions [adjusted odds ratio (AOR) = 0.17, 95% CI 0.15–0.20], 92%-reduced odds of local adverse reactions (AOR = 0.08, 95% CI 0.06–0.09), and 76%-reduced odds of systemic adverse reactions (AOR = 0.24, 95% CI 0.16–0.28). Sensitivity analysis using propensity score matching and inverse probability of treatment weighting suggested highly consistent results (see **eTable 2 and eTable 3**). Extended models testing for the interaction between potential effect modifiers yielded no statistically significant results ($P > 0.05$).

Table 3 shows the adjusted odds ratios of adverse reactions following the second dose compared with the first. For adverse reactions of any type, there were 18%-increased odds (AOR = 1.18, 95% CI 1.01–1.37) for the second dose compared with the first among those receiving CoronaVac. Among those receiving Comirnaty, there were 106% increased odds (AOR = 2.06, 95% CI 1.81–2.35). For all three sub-types of adverse reactions, significantly increased odds were observed in the Comirnaty group. Among those receiving CoronaVac, significantly increased odds were only observed for local adverse reactions.

4. Discussion

The results confirmed our hypothesis that CoronaVac had milder reactogenicity compared with Comirnaty. We found that the risk of adverse reactions (overall, local, and systemic) two weeks post-vaccination is significantly lower among those receiving CoronaVac compared with Comirnaty. This risk difference does not vary significantly between those living with multimorbidity

Table 1
Cohort characteristics.

	CoronaVac	Comirnaty		
n	1129	969	Standardized mean difference	
Age (mean (SD))	46.49 (14.42)	43.13 (16.54)	0.217	***
Sex = Male (%)	527 (46.7)	498 (51.7)	0.101	*
Educational attainment (%)			0.301	***
Primary and below	20 (1.8)	27 (2.8)		
Secondary	373 (33)	215 (22.2)		
Post-secondary	176 (15.6)	116 (12)		
University or above	560 (49.6)	610 (63)		
Smoking status (%)			0.172	**
Non-smoker	974 (86.3)	888 (91.6)		
Former smoker	40 (3.5)	24 (2.5)		
Current smoker	114 (10.1)	57 (5.9)		
Alcohol use (%)			0.144	*
Non-drinker	807 (71.5)	632 (65.4)		
Occasional drinker	223 (19.8)	221 (22.9)		
Former drinker	5 (0.4)	3 (0.3)		
Regular drinker	94 (8.3)	111 (11.5)		
Number of chronic medications (%)			0.147	*
None	917 (81.2)	761 (78.5)		
1–2	155 (13.7)	155 (16)		
3–4	40 (3.5)	39 (4)		
5–9	13 (1.2)	14 (1.4)		
10 or more	4 (0.4)	0 (0)		
History of allergy to medications (%)	82 (7.3)	56 (5.8)	0.059	
History of allergy to food and other substances (%)	70 (6.2)	65 (6.7)	0.022	
Chronic conditions (%)				
Asthma	10 (0.9)	18 (1.9)	0.084	
Psoriasis	0 (0)	1 (0.1)	0.045	
Rheumatoid arthritis	0 (0)	3 (0.3)	0.079	
Systemic lupus erythematosus	1 (0.1)	0 (0)	0.042	
Cancer remission	8 (0.7)	4 (0.4)	0.040	
Cancer under treatment	1 (0.1)	4 (0.4)	0.065	
Hypertension	102 (9)	100 (10.3)	0.043	
Hypercholesterolemia	81 (7.2)	74 (7.6)	0.018	
Heart disease	18 (1.6)	16 (1.7)	0.004	
Diabetes	32 (2.8)	35 (3.6)	0.044	
Stroke	2 (0.2)	3 (0.3)	0.027	
Neurological disorder	1 (0.1)	2 (0.2)	0.031	
Mental health disorder	10 (0.9)	8 (0.8)	0.007	
Liver problems	6 (0.5)	10 (1)	0.057	
Kidney problems	3 (0.3)	5 (0.5)	0.040	
Morbidity status (%)			0.076	
No chronic conditions	935 (82.8)	778 (80.3)		
One	132 (11.7)	124 (12.8)		
Two	46 (4.1)	47 (4.9)		
Three	12 (1.1)	16 (1.7)		
Four or more	4 (0.4)	4 (0.4)		

*** P < 0.05, ** P < 0.01, * P < 0.001

and those without, between men and women, and between older and non-older adults in our cohort. We also observed a higher risk of adverse reactions following the second dose compared with the first, with larger differences among those receiving Comirnaty. Our findings may further inform individual and public choices of vaccines.

Post-marketing research on Covid-19 vaccines in real-world settings is still accruing, with most studies focusing on serious adverse events which typically require medical interventions or even tertiary care.[20] While this line of research is highly important to establish the safety profile, the reactogenicity of vaccines, represented by adverse reactions that are mild and oftentimes fully self-resolves, also has a considerable impact on individual and public decisions with regard to vaccine uptake [21]. To the best of our knowledge, this current post-marketing study is the first to compare the reactogenicity of CoronaVac with Comirnaty in the same population. Our findings are in line with previous clinical trial data [10,14]. For instance, the recently published phase III clinical trial results suggested that approximately one-fifth of the volunteers receiving CoronaVac experienced any type of adverse reactions [14] and approximately 80% of individuals receiving Comirnaty reported adverse reactions after both doses, such as pain at the injection site, in the first seven days [10].

Recently published data obtained from vaccinated healthcare workers in Hong Kong suggested that, compared with Comirnaty, the quantity of antibodies induced in adults receiving CoronaVac is substantially lower [22]. Also, it has been suggested in a *meta*-analysis that, across different vaccine platforms, there are obvious trade-offs between various qualities of the vaccines including mild reactogenicity and the strength of the triggered immune response [11]. It is possible that the general immune response induced by vaccination was weaker among those receiving CoronaVac, compared with those receiving Comirnaty, and thus potentially a lower risk of adverse reactions followed the vaccination of the participants; further immunoepidemiologic studies are needed to test this hypothesis because there is no direct relationship between side effects and protection.

Given the real-world observational design, randomization was not feasible to further eliminate any residual confounding effects beyond the multivariable adjustment made in the models. Specif-

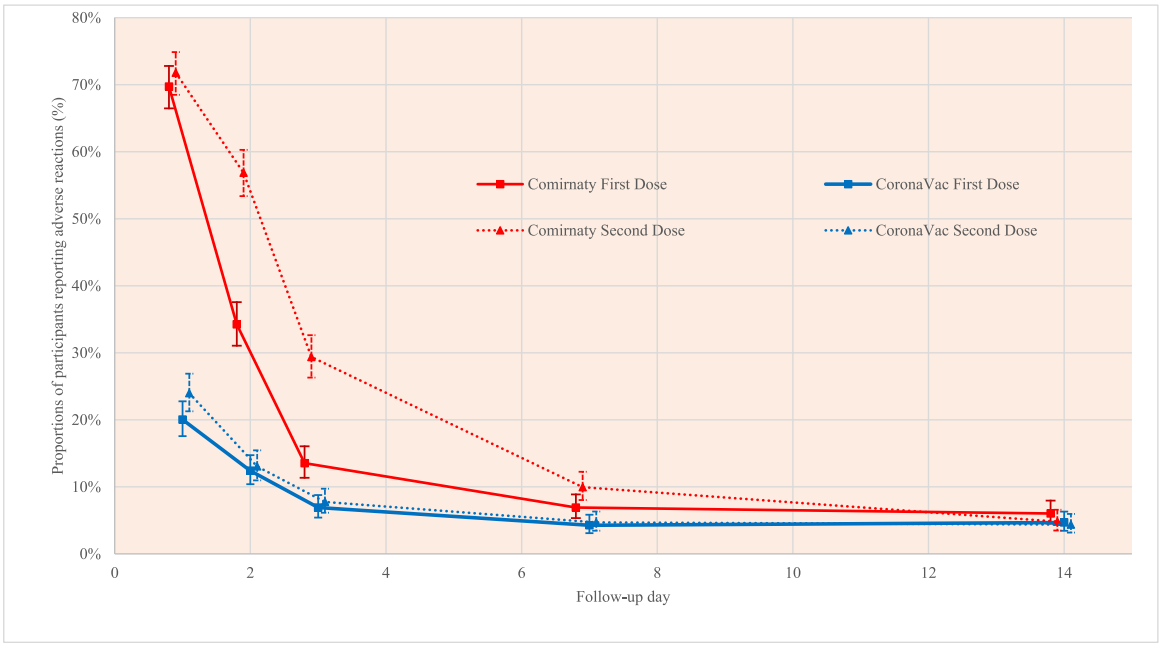


Fig. 1. Proportions (with 95% confidence intervals) of self-reported adverse reactions by vaccine type and dose (first versus second). Sample size varies across timepoints with different retention rate on different follow-up days.

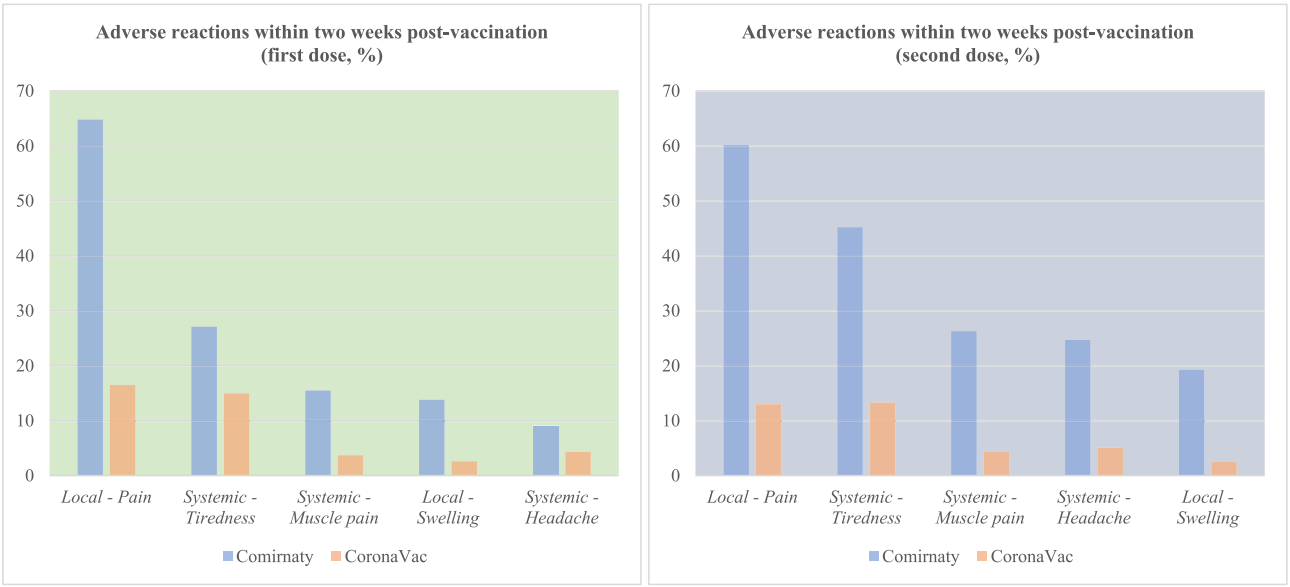


Fig. 2. Proportions of participants reporting specific adverse reactions two weeks post-vaccination.

ically, there could be unobserved characteristics of individuals that were associated with the choice of vaccine type and, simultaneously, with self-reports of adverse reactions, such that the results were biased towards the rejection of the null hypothesis. Nonetheless, based on our literature search and clinical reasoning we did not identify any further potential confounders to consider and include in the analysis. Besides residual confounding, other limitations that need to be taken into consideration while interpreting the results include the design of serial self-report online survey, which entails a risk of omitting the follow-up survey of individuals (from the missing follow-up data) who had more serious adverse reactions and required medical interventions or were even hospitalized. However, both vaccine groups had a response rate of > 80% throughout the follow-up period and any bias should not affect the results and conclusions substantially. Also, more serious adverse reactions, if any, would most likely be captured in the routine medical databases which are closely monitored and reported. In addition, this study lacked the clinical confirmation of the adverse reactions and the causality assessment which would have strengthened the causal inferences from the observed associations.

Previous research on vaccine hesitancy suggested that reactogenicity is among the multitude of factors considered while making the decision to receive a vaccine or not [23]. A clearer outline

Table 2
Adjusted odds ratios [a] of self-reported adverse reactions for those who received CoronaVac compared with those receiving Comirnaty.

	Odds ratio (95% confidence interval)
Adverse reactions	
Any	0.17 (0.15–0.20) ***
Local [b]	0.08 (0.06–0.09) ***
Systemic [c]	0.24 (0.16–0.28) ***
Severe allergic reactions [d]	0.62 (0.36–1.06)

*** P < 0.05, ** P < 0.01, * P < 0.001

[a] Odds ratios adjusted for dose (1st versus 2nd), follow-up day, age, sex, educational attainment, allergy to medications, allergy to food and other substances, smoking status, alcohol use, number of chronic medications, ankylosing spondylitis, asthma, psoriasis, rheumatoid arthritis, systemic lupus erythematosus, cancer remission, cancer under treatment, hypertension, hypercholesterolemia, heart disease, diabetes, stroke, neurological disorders, mental health disorders, liver problems, and kidney problems

[b] Including numbness, soreness, pain, swelling, redness, and itch

[c] Including sore throat, tiredness, fever, chills, sweating, cough, headache, muscle pain, joint pain, pain in limbs, abdominal pain, diarrhea, nausea, vomiting, poor appetite, insomnia, feeling unwell, enlarged lymph nodes, rash, and temporary one-sided facial drooping

[d] Including hypotension, dizziness, itchy skin rash, swelling of face or tongue, and wheezing/shortness of breath

Table 3
Adjusted odds ratios [a] of self-reported adverse reactions arising from the second dose compared with the first dose of CoronaVac and Comirnaty.

	Odds ratio (95% confidence interval)	
	CoronaVac	Comirnaty
Adverse reactions		
Any	1.18 (1.01–1.37) *	2.06 (1.81–2.35) ***
Local [b]	1.39 (1.11–1.75) **	2.04 (1.77–2.36) ***
Systemic [c]	1.12 (0.92–1.38)	3.09 (2.65–3.61) ***
Severe allergic reactions [d]	1.15 (0.62–2.15)	2.01 (1.21–3.33) **

*** P < 0.05, ** P < 0.01, * P < 0.001

[a] Odds ratios adjusted for follow-up day, age, sex, educational attainment, allergy to medications, allergy to food and other substances, smoking status, alcohol use, number of chronic medications, ankylosing spondylitis (only for CoronaVac), asthma, psoriasis (only for Comirnaty), rheumatoid arthritis (only for Comirnaty), systemic lupus erythematosus (only for CoronaVac), cancer remission, cancer under treatment, hypertension, hypercholesterolemia, heart disease, diabetes, stroke, neurological disorders, mental health disorders, liver problems, and kidney problems

[b] Including numbness, soreness, pain, swelling, redness, and itch

[c] Including sore throat, tiredness, fever, chills, sweating, cough, headache, muscle pain, joint pain, pain in limbs, abdominal pain, diarrhea, nausea, vomiting, poor appetite, insomnia, feeling unwell, enlarged lymph nodes, rash, and temporary one-sided facial drooping

[d] Including hypotension, dizziness, itchy skin rash, swelling of face or tongue, and wheezing/shortness of breath

of the types of anticipated adverse reactions following vaccination should enable more informed decisions for both individuals and governments. Specifically, our study findings should help shape the public's expectation of the reactogenicity of CoronaVac, as compared with the more widely investigated Comirnaty [24]. Vaccination or medical leave policies could be formulated on the basis of our findings. Nevertheless, further research in other populations is warranted to verify our results and test for generalizability. The Government of Hong Kong continues to monitor all serious adverse events following immunization (AEFI). To date, there have not been major safety signals on serious AEFI. However, successful infection control and risk mitigation strategies against Covid-19 [25] has led to a very low COVID-19 infection rate in Hong Kong (<12,000 cases in a population of over seven million people as of July 2021). In this context, the self-reported adverse reactions of vaccines become an important factor in the decision of vaccine uptake.

In conclusion, this first post-marketing study comparing the reactogenicity of CoronaVac and Comirnaty in the same population suggests a lower risk of self-reported adverse reactions following vaccination with CoronaVac compared with Comirnaty.

Funding

ICKW received funding for this work from the Food and Health Bureau of the Hong Kong Special Administration Region Government, Hong Kong, China (Ref: COVID19F01) Funder website: https://www.fhb.gov.hk/en/index.html. The funder had no role in study design, data collection and analysis, and decision to publish.

Data availability statement

Authorization to access the data may be considered by the authors upon reasonable requests. Requests to access these datasets should be directed to the corresponding author, ewchan@hku.hk.

6. Ethics approval

This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW-21–090) and the Department of Health Ethics Committee (LM 21/2021).

Informed consent

Upon recruitment, written informed consent from the participants were obtained. The consent form, patient information leaflet, paper questionnaires can be downloaded from our website (https://www.hkcare.hku.hk/).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Ian Chi Kei Wong reports financial support was provided by Food and Health Bureau of the Hong Kong Special Administration Region Government. Esther Wai Yin Chan reports a relationship with Hospital Authority that includes: consulting or advisory and speaking and lecture fees. Esther Wai Yin Chan reports a relationship with Research Grants Council (RGC, Hong Kong) that includes: funding grants. Esther Wai Yin Chan reports a relationship with Research Fund Secretariat of the Food and Health Bureau that includes: funding grants. Esther Wai Yin Chan reports a relationship with National Natural Science Fund of China that includes: funding grants. Esther Wai Yin Chan reports a relationship with Wellcome Trust that includes: funding grants. Esther Wai Yin Chan reports a relationship with Bayer that includes: funding grants. Esther Wai Yin Chan reports a relationship with Bristol-Myers Squibb that includes: funding grants. Esther Wai Yin Chan reports a relationship with Pfizer that includes: funding grants. Esther Wai Yin Chan reports a relationship with Janssen that includes: funding grants. Esther Wai Yin Chan reports a relationship with Amgen that includes: funding grants. Esther Wai Yin Chan reports a relationship with Takeda that includes: funding grants. Esther Wai Yin Chan reports a relationship with Narcotics Division of the Security Bureau of HKSAR that includes: funding grants. Francisco Tsz Tsun Lai reports a relationship with RGC Postdoctoral Fellowship, Hong Kong Research Grants Council that includes: funding grants. Xue Li reports a relationship with Food and Health Bureau of the Government of the Hong Kong SAR that includes: funding grants. Xue Li reports a relationship with Janssen that includes: funding grants. Xue Li reports a relationship with Pfizer that includes: funding grants. Xue Li reports a relationship with The University of Hong Kong that includes: funding grants. Xue Li reports a relationship with Merck Sharp & Dohme that includes: consulting or advisory. Celine Sze Ling Chui reports a relationship with Food and Health

Bureau of the Hong Kong Government that includes: funding grants. Celine Sze Ling Chui reports a relationship with Hong Kong Research Grant Council that includes: funding grants. Celine Sze Ling Chui reports a relationship with Hong Kong Innovation and Technology Commission that includes: funding grants. Celine Sze Ling Chui reports a relationship with Pfizer that includes: funding grants. Celine Sze Ling Chui reports a relationship with IQVIA that includes: funding grants. Celine Sze Ling Chui reports a relationship with Amgen that includes: funding grants. Celine Sze Ling Chui reports a relationship with PrimeVigilance Ltd that includes: consulting or advisory. Eric Yuk Fai Wan reports a relationship with Food and Health Bureau of the Government of the Hong Kong SAR that includes: funding grants. Eric Yuk Fai Wan reports a relationship with Hong Kong Research Grants Council that includes: funding grants. Ian Chi Kei Wong reports a relationship with Amgen that includes: funding grants. Ian Chi Kei Wong reports a relationship with Bristol-Myers Squibb that includes: funding grants. Ian Chi Kei Wong reports a relationship with Pfizer that includes: funding grants. Ian Chi Kei Wong reports a relationship with Janssen that includes: funding grants. Ian Chi Kei Wong reports a relationship with Bayer that includes: funding grants. Ian Chi Kei Wong reports a relationship with GSK that includes: funding grants. Ian Chi Kei Wong reports a relationship with Novartis that includes: funding grants. Ian Chi Kei Wong reports a relationship with Hong Kong RGC that includes: funding grants. Ian Chi Kei Wong reports a relationship with Hong Kong Health and Medical Research Fund that includes: funding grants. Ian Chi Kei Wong reports a relationship with National Institute for Health Reseaorch in England that includes: funding grants. Ian Chi Kei Wong reports a relationship with European Commission that includes: funding grants. Ian Chi Kei Wong reports a relationship with National Health and Medical Research Council in Australia that includes: funding grants. Ian Chi Kei Wong reports a relationship with Janssen that includes: speaking and lecture fees. Ian Chi Kei Wong reports a relationship with Medice that includes: speaking and lecture fees.].

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2022.01.062.

References

[1] World Health Organization. Interim recommendations for use of the inactivated COVID-19 vaccine, CoronaVac, developed by Sinovac [Internet]. 2021 [cited 2021 Jul 18]. Available from: https://apps.who.int/iris/rest/bitstreams/1348680/retrieve

[2] Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021;21(2):181–92.

[3] Tanriover MD, Doğanay HL, Akova M, Güner HR, Azap A, Akhan S, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. The Lancet 2021;398(10296):213–22.

[4] Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. N Engl J Med 2021;385(10):875–84.

[5] Mathioudakis AG, Ghrew M, Ustianowski A, Ahmad S, Borrow R, Papavasileiou LP, et al. Self-Reported Real-World Safety and Reactogenicity of COVID-19 Vaccines: A Vaccine Recipient Survey. Life (Basel, Switzerland). 2021;11.

[6] McDonald I, Murray SM, Reynolds CJ, Altmann DM, Boyton RJ. Comparative systematic review and meta-analysis of reactogenicity. immunogenicity and efficacy of vaccines against SARS-CoV-2 npj Vaccines 2021;6(1). https://doi.org/10.1038/s41541-021-00336-1.

[7] Chodick G, Tene L, Rotem RS, Patalon T, Gazit S, Ben-Tov A, et al. The Effectiveness of the Two-Dose BNT162b2 Vaccine: Analysis of Real-World Data. Clin Infect Dis 2021. https://doi.org/10.1093/cid/ciab438.

[8] Robertson E, Reeve KS, Niedzwiedz CL, Moore J, Blake M, Green M, et al. Predictors of COVID-19 vaccine hesitancy in the UK household longitudinal study. Brain Behav Immun [Internet]. 2021;94:41–50.

[9] Walsh EE, Frenck RW, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med 2020;383(25):2439–50.

[10] Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020;383(27):2603–15.

[11] Gringeri M, Mosini G, Battini V, Cammarata G, Guarnieri G, Carnovale C, et al. Preliminary evidence on the safety profile of BNT162b2 (Comirnaty): new insights from data analysis in EudraVigilance and adverse reaction reports from an Italian health facility. Human Vaccines & Immunotherapeutics 2021;17(9):2969–71.

[12] Borobia AM, Carcas AJ, Pérez-Olmeda M, Castaño L, Bertran MJ, García-Pérez J, et al. Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (CombiVacS): a multicentre, open-label, randomised, controlled, phase 2 trial. Lancet [Internet]. 2021;398:121–30. Available from https://www.sciencedirect.com/science/article/pii/S0140673621014203.

[13] Krantz MS, Kwah JH, Stone CA, Phillips EJ, Ortega G, Banerji A, et al. Safety Evaluation of the Second Dose of Messenger RNA COVID-19 Vaccines in Patients With Immediate Reactions to the First Dose. JAMA Intern Med 2021;181(11):1530. https://doi.org/10.1001/jamainternmed.2021.3779.

[14] Tanriover MD, Doğanay HL, Akova M, Güner HR, Azap A, Akhan S, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. Lancet. Elsevier 2021;398:213–22.

[15] Al Kaabi N, Zhang Y, Xia S, Yang Y, Al Qahtani MM, Abdulrazzaq N, et al. Effect of 2 Inactivated SARS-CoV-2 Vaccines on Symptomatic COVID-19 Infection in Adults: A Randomized Clinical Trial. JAMA 2021;326(1):35. https://doi.org/10.1001/jama.2021.8565.

[16] Yu Y, Lau JTF, Lau MMC, Wong MCS, Chan PKS. Understanding the Prevalence and Associated Factors of Behavioral Intention of COVID-19 Vaccination Under Specific Scenarios Combining Effectiveness, Safety, and Cost in the Hong Kong Chinese General Population. Int J Health Policy Manag 2021. https://doi.org/10.34172/ijhpm.2021.02.

[17] Barnett K, Mercer SW, Norbury M, Watt G, Wyke S, Guthrie B. Epidemiology of multimorbidity and implications for health care, research, and medical education: a cross-sectional study 2012;380:37–43.

[18] Huntley AL, Johnson R, Purdy S, Valderas JM, Salisbury C. Measures of multimorbidity and morbidity burden for use in primary care and community settings: A systematic review and guide. Ann. Fam. Med. 2012;10(2):134–41.

[19] van Smeden M, Moons KGM, de Groot JAH, Collins GS, Altman DG, Eijkemans MJC, et al. Sample size for binary logistic prediction models: Beyond events per variable criteria. Stat Methods Med Res 2019;28(8):2455–74.

[20] Dagan N, Barda N, Kepten E, Miron O, Perchik S, Katz MA, et al. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. N Engl J Med 2021;384(15):1412–23.

[21] Finney Rutten LJ, Zhu X, Leppin AL, Ridgeway JL, Swift MD, Griffin JM, et al. Evidence-Based Strategies for Clinical Organizations to Address COVID-19 Vaccine Hesitancy. Mayo Clin Proc [Internet]. 2021;96(3):699–707.

[22] Lim WW, Mak L, Leung GM, Cowling BJ, Peiris M. Comparative immunogenicity of mRNA and inactivated vaccines against COVID-19. The Lancet Microbe 2021;2(9):e423. https://doi.org/10.1016/S2666-5247(21)00177-4.

[23] Rief W. Fear of Adverse Effects and COVID-19 Vaccine Hesitancy: Recommendations of the Treatment Expectation Expert Group. JAMA Health Forum 2021;2(4):e210804. https://doi.org/10.1001/jamahealthforum.2021.0804.

[24] Fabiani M, Ramigni M, Gobbetto V, Mateo-Urdiales A, Pezzotti P, Piovesan C. to 24 March 2021. Eurosurveillance [Internet]. 2021;26(17). https://doi.org/10.2807/1560-7917.ES.2021.26.17.2100420.

[25] Wong SYS, Kwok KO, Chan FKL. What can countries learn from Hong Kong's response to the COVID-19 pandemic? CMAJ [Internet]. CMAJ 2020;192:E511–5. Available from https://www.cmaj.ca/content/192/19/E511.

CoronaVac

O que a ciência comprova

8.4. CoronaVac é a vacina com menos efeitos adversos dentre as que estão em uso no Brasil, revelam estudos

Um estudo publicado na revista científica Lancet Infectious Diseases revelou que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, causa efeitos adversos em apenas 29% a 33% dos vacinados, e todos muito leves (como dor no braço ou fadiga passageira). Esse é um ótimo indicador, que atesta o alto perfil de segurança do imunizante, e um dos menores índices de efeitos adversos entre todas as vacinas aprovadas até o momento para uso emergencial pela Organização Mundial de Saúde.

O estudo foi realizado por pesquisadores dos Centros de Controles de Doenças das províncias de Hangzhou, Nanjing e Jiangsu, na China, cientistas da Academia Chinesa de Ciências e pesquisadores da Sinovac, com 744 voluntários que participaram dos ensaios clínicos de fase 1 e 2 da CoronaVac. Na fase 1, 29% dos voluntários relataram ter experimentado reações adversas, principalmente dor no local da aplicação e fadiga, no período de 14 dias após receber a vacina. Na fase 2, apenas 33% dos voluntários relataram efeitos adversos. Menos de 5% dos voluntários em ambas as etapas tiveram sintomas de febre, dor de cabeça ou náusea.

No Brasil, dados sobre a segurança da vacina do Butantan foram obtidos em ensaios clínicos de fase 3 com 9 mil voluntários em 2020. As manifestações indesejadas foram muito leves e não foi necessária atenção médica maior. No Projeto S, estudo clínico realizado pelo Butantan na cidade de Serrana, foram administradas 54.882 doses na população adulta do município e não houve relato de evento adverso grave relacionado à vacinação. Durante a aplicação da primeira dose do imunizante em Serrana, foram 4,4% de relatos de reações adversas e apenas 0,02% considerados de grau 3 (mialgia e cefaleia), porque interferiram nas atividades diárias. Já na segunda dose, houve somente 0,2% de relatos de efeitos adversos, nenhum considerado de grau 3 ou superior. Outro indicador que atesta a segurança da CoronaVac é que, até hoje, a área de Farmacovigilância

do Butantan não recebeu nenhum relato de trombose associado à vacinação – um dos efeitos adversos já relatados em outras vacinas contra a Covid-19.

Tais resultados contrastam com as conclusões observadas em estudos com as demais vacinas contra a Covid-19 – embora não seja possível comparar diretamente os resultados de pesquisas, pois os grupos estudados são diferentes, assim como as metodologias de análise. Entre 70% e 75% dos norte-americanos que tomaram vacinas feitas com a tecnologia do RNA mensageiro (mRNA) relataram experimentar efeitos adversos, percentual que subiu para 86% a 88% entre pacientes britânicos que tomaram a vacina AstraZeneca/Oxford, feita com a tecnologia de vetor viral. Já no caso da vacina da Janssen, também de vetor viral, entre 35% e 62% dos entrevistados relataram efeitos adversos.

A tecnologia empregada na CoronaVac, de vírus inativado, é uma das mais estudadas e seguras do mundo. O vírus é replicado e, posteriormente, morto. Assim, não é capaz de se multiplicar no corpo e adoecer o organismo, mas consegue desencadear a produção de anticorpos e produzir resposta imunológica.

Vacinas feitas com a tecnologia do RNA mensageiro (mRNA)

Um estudo publicado no jornal da Associação Americana de Medicina em abril de 2021 sobre a percepção de efeitos adversos das vacinas das farmacêuticas americanas Pfizer ou Moderna, produzidas com a tecnologia do RNA mensageiro (mRNA), foi feito com 3,6 milhões de norte-americanos que tomaram a primeira dose, e 1,9 milhão que tomaram a segunda dose. A maioria dos participantes relatou ter experimentado reação no local da injeção (70% dos que tomaram a primeira dose, e 75% dos que receberam a segunda dose) ou reação sistêmica (50% após a primeira dose, e 69,4% após a segunda dose) durante os primeiros sete

dias após a vacinação. As reações mais frequentes após a primeira dose da vacina foram dor no local da injeção (67,8%), fadiga (30,9%), cefaleia (25,9%) e mialgia (19,4%). O relato de efeitos adversos foi maior após a segunda dose para ambas as vacinas, particularmente para reações como fadiga (53,9%), dor de cabeça (46,7%), mialgia (44%), calafrios (31,3%), febre (29,5%) e dos nas articulações (25,6%).

Vacinas feitas com vetor viral

Um estudo publicado na The Lancet em novembro de 2020 analisou a percepção de efeitos adversos de 560 adultos que receberam a vacina elaborada pela farmacêutica anglo-sueca AstraZeneca e por pesquisadores da Universidade Oxford. Entre aqueles que receberam duas doses, após a primeira dose foram relatadas reações locais em 88% dos participantes no grupo de 18 a 55 anos, 73% no grupo de 56 e 69 anos, e 61% no grupo de 70 anos e mais. Foram relatadas reações sistêmicas em 86% dos participantes no grupo de 18 a 55 anos, 77% no grupo de 56 a 69 anos, e 65% no grupo de 70 anos ou mais. Fadiga, dor de cabeça, febre e mialgia foram as reações adversas sistêmicas mais comumente relatadas.

Além disso, o Centro de Controle de Doenças dos Estados Unidos realizou um levantamento em agosto de 2021 com 3.356 norte-americanos que tomaram a dose única da vacina da farmacêutica Janssen. No grupo de 18 a 59 anos, um total de 62% relataram ter experimentado um ou mais efeitos adversos, sendo os principais deles fadiga (43,8%), dor de cabeça (44,4%), mialgia (39,1%), náusea (15,5%) e febre (12,8%). Já no grupo com mais de 60 anos, 35% tiveram algum efeito adverso, como fadiga (29,7%), dor de cabeça (30,4%), mialgia (24%), náusea (10,8%) e febre (3,1%).

Publicado em: 17/11/2020

Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial

Yanjun Zhang, Gang Zeng*, Hongxing Pan*, Changgui Li*, Yaling Hu, Kai Chu, Weixiao Han, Zhen Chen, Rong Tang, Weidong Yin, Xin Chen, Yuansheng Hu, Xiaoyong Liu, Congbing Jiang, Jingxin Li, Minnan Yang, Yan Song, Xiangxi Wang, Qiang Gao†, Fengcai Zhu†*

Summary

Background With the unprecedented morbidity and mortality associated with the COVID-19 pandemic, a vaccine against COVID-19 is urgently needed. We investigated CoronaVac (Sinovac Life Sciences, Beijing, China), an inactivated vaccine candidate against COVID-19, containing inactivated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), for its safety, tolerability and immunogenicity.

Methods In this randomised, double-blind, placebo-controlled, phase 1/2 clinical trial, healthy adults aged 18–59 years were recruited from the community in Suining County of Jiangsu province, China. Adults with SARS-CoV-2 exposure or infection history, with axillary temperature above 37·0°C, or an allergic reaction to any vaccine component were excluded. The experimental vaccine for the phase 1 trial was manufactured using a cell factory process (CellSTACK Cell Culture Chamber 10, Corning, Wujiang, China), whereas those for the phase 2 trial were produced through a bioreactor process (ReadyToProcess WAVE 25, GE, Umea, Sweden) . The phase 1 trial was done in a dose-escalating manner. At screening, participants were initially separated (1:1), with no specific randomisation, into two vaccination schedule cohorts, the days 0 and 14 vaccination cohort and the days 0 and 28 vaccination cohort, and within each cohort the first 36 participants were assigned to block 1 (low dose CoronaVac [3 μg per 0·5 mL of aluminium hydroxide diluent per dose) then another 36 were assigned to block 2 (high-dose Coronavc [6 μg per 0·5 mL of aluminium hydroxide diluent per dse]). Within each block, participants were randomly assigned (2:1), using block randomisation with a block size of six, to either two doses of CoronaVac or two doses of placebo. In the phase 2 trial, at screening, participants were initially separated (1:1), with no specific randomisation, into the days 0 and 14 vaccination cohort and the days 0 and 28 vaccination cohort, and participants were randomly assigned (2:2:1), using block randomisation with a block size of five, to receive two doses of either low-dose CoronaVac, high-dose CoronaVac, or placebo. Participants, investigators, and laboratory staff were masked to treatment allocation. The primary safety endpoint was adverse reactions within 28 days after injection in all participants who were given at least one dose of study drug (safety population). The primary immunogenic outcome was seroconversion rates of neutralising antibodies to live SARS-CoV-2 at day 14 after the last dose in the days 0 and 14 cohort, and at day 28 after the last dose in the days 0 and 28 cohort in participants who completed their allocated two-dose vaccination schedule (per-protocol population). This trial is registered with ClinicalTrials.gov, NCT04352608, and is closed to accrual.

Findings Between April 16 and April 25, 2020, 144 participants were enrolled in the phase 1 trial, and between May 3 and May 5, 2020, 600 participants were enrolled in the phase 2 trial. 743 participants received at least one dose of investigational product (n=143 for phase 1 and n=600 for phase 2; safety population). In the phase 1 trial, the incidence of adverse reactions for the days 0 and 14 cohort was seven (29%) of 24 participants in the 3 ug group, nine (38%) of 24 in the 6 μg group, and two (8%) of 24 in the placebo group, and for the days 0 and 28 cohort was three (13%) of 24 in the 3 μg group, four (17%) of 24 in the 6 μg group, and three (13%) of 23 in the placebo group. The seroconversion of neutralising antibodies on day 14 after the days 0 and 14 vaccination schedule was seen in 11 (46%) of 24 participants in the 3 μg group, 12 (50%) of 24 in the 6 μg group, and none (0%) of 24 in the placebo group; whereas at day 28 after the days 0 and 28 vaccination schedule, seroconversion was seen in 20 (83%) of 24 in the 3 μg group, 19 (79%) of 24 in the 6 μg group, and one (4%) of 24 in the placebo group. In the phase 2 trial, the incidence of adverse reactions for the days 0 and 14 cohort was 40 (33%) of 120 participants in the 3 μg group, 42 (35%) of 120 in the 6 μg group, and 13 (22%) of 60 in the placebo group, and for the days 0 and 28 cohort was 23 (19%) of 120 in the 3 μg group, 23 (19%) of 120 in the 6 μg group, and 11 (18%) of 60 for the placebo group. Seroconversion of neutralising antibodies was seen for 109 (92%) of 118 participants in the 3 μg group, 117 (98%) of 119 in the 6 μg group, and two (3%) of 60 in the placebo group at day 14 after the days 0 and 14 schedule; whereas at day 28 after the days 0 and 28 schedule, seroconversion was seen in 114 (97%) of 117 in the 3 μg group, 118 (100%) of 118 in the 6 μg group, and none (0%) of 59 in the placebo group.

Lancet Infect Dis 2021; 21: 181–92

Published Online November 17, 2020 https://doi.org/10.1016/S1473-3099(20)30843-4

See Comment page 150

For the Chinese translation of the abstract see Online for appendix 1

*Joint first authors

†Contributed equally

Department of Microbiology, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China (Prof Y Zhang PhD); **Sinovac Biotech, Beijing, China** (G Zeng PhD, Ya Hu MSc, W Han MSc, W Yin, MBA, Yu Hu MPH); **Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China** (H Pan MSc, K Chu MSc, R Tang BA, Prof J Li PhD, Prof F Zhu MD); **National Institutes for Food and Drug Control, Beijing, China** (Prof C Li PhD, Z Chen MSc); **Suining County Center for Disease Control and Prevention, Suining, Jiangsu Province, China** (X Chen BA, X Liu BA, C Jiang BA, Y Song BA); **CAS Key Laboratory of Infection and Immunity, National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China** (M Yang PhD, Prof X Wang PhD) **and Sinovac Life Sciences, Beijing, China** (Q Gao MSc)

Correspondence to:
Dr Qiang Gao, Sinovac Life Sciences, Beijing 100085, China
gaoq@sinovac.com

or

Dr Fengcai Zhu, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210009, China
jszfc@vip.sina.com

Interpretation Taking safety, immunogenicity, and production capacity into account, the 3 µg dose of CoronaVac is the suggested dose for efficacy assessment in future phase 3 trials.

Funding Chinese National Key Research and Development Program and Beijing Science and Technology Program.

Introduction

The on-going COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to high morbidity and mortality worldwide.[1] Globally, as of Oct 28, 2020, 43·3 million laboratory-confirmed cases of SARS-CoV-2 infection have been reported, resulting in 1·15 million deaths.[2]

Although physical distancing, quarantine, and isolation were effective in limiting the number of people becoming infected during the pandemic in the short term, the absence of immunity in the population leave them susceptible to further waves of SARS-CoV-2 infection. Health-care workers, older people (aged >60 years), and those with underlying health conditions are at particularly high risk.[3,4] The shortage of an effective treatment for COVID-19 has led to quick action in the development of potential vaccines against the disease.

Since the outbreak began, researchers around the world have been trying to develop vaccines for COVID-19, with more than 198 vaccines currently in preclinical or clinical development.[5] Frenetic efforts towards the development of a vaccine have led to several candidate vaccines, derived from multiple platforms and progressing to the clinical evaluation stage, including inactivated vaccines, live virus vaccines, recombinant protein vaccines, vectored vaccines, and DNA or RNA vaccines.[6–14] Development of various vaccine platforms and strategies in parallel is essential because little is known of the nature of protective immune responses to COVID-19 and which vaccine strategies will be most successful is unclear.

CoronaVac (Sinovac Life Sciences, Beijing, China) is an inactivated vaccine candidate against COVID-19 that has shown good immunogenicity in mice, rats, and non-human primates with vaccine-induced neutralising antibodies to SARS-CoV-2, which could neutralise ten representative strains of SARS-CoV-2.[15] Moreover, the results indicated CoronaVac provided partial or complete protection in macaques from severe interstitial pneumonia after a SARS-CoV-2 challenge, without observable antibody-dependent enhancement of infection, which support progression to clinical trials in humans.[15]

Methods

Study design and participants

In this single-centre, double-blind, randomised, placebo-controlled, phase 1/2 clinical trial, participants were recruited from the community to assess two two-dose regimens of CoronaVac. The study was run at Jiangsu Provincial Center for Disease Control and Prevention (CDC) in Suining County, Jiangsu province, China. The phase 1 trial was dose-escalation study. In phase 1, participants were recruited and allocated sequentially

Research in context

Evidence before this study

We searched PubMed and the American Medical Association website on Aug 13, 2020, for published research articles, with no language or date restrictions, using the search terms of "SARS-CoV-2", "COVID-19", "vaccine", and "clinical trial". The search results showed that the COVID-19 pandemic resulted in an unprecedented race to develop an effective vaccine. We identified preclinical data on three immunisations using two different doses of CoronaVac (3 µg and 6 µg per dose), an inactivated whole virus vaccine against COVID-19 developed by Sinovac Life Sciences (Beijing, China), providing partial or complete protection in macaques against SARS-CoV-2 challenge, without observable antibody-dependent enhancement of infection. We also identified a phase 2 clinical trial of another inactivated vaccine developed by Sinopharm (Beijing, China), which showed the incidence of adverse reactions was 19·0% within 28 days after two doses of vaccine (5 µg in 0·5 mL of diluent) in a day 0 and 21 vaccination schedule, and the seroconversion rates of the neutralising antibody detected by plaque reduction neutralisation test was 97·6% at 14 days after a day 0 and 21 vaccination schedule. The clinical study of CoronaVac can further provide safety and immunogenic evidence for the inactivated vaccine.

Added value of this study

In this first in-human study of CoronaVac, we used a phase 1/2 study design to screen the safety of two doses and two vaccination schedules in a dose-escalation study in a small cohort before expanding the study to a larger cohort to explore the immunogenicity of the vaccine in healthy adults. The immune response in the phase 2 study was substantially higher than in the phase 1 study, which might be due to the difference in preparation process of vaccine batches used in phase 1 and 2 resulting in a higher proportion of intact spike protein on the purified inactivated SARS-CoV-2 virions in the vaccine used in phase 2 than that used in phase 1.

Implications of all the available evidence

Data from this study support the approval of emergency use of CoronaVac in China, and three phase 3 clinical trials that are ongoing in Brazil, Indonesia, and Turkey.

(1:1), with no specific randomisation, to one of two vaccination schedules, with either a 14-day interval (the day 0 and 14 vaccination cohort) or a 28-day interval (the day 0 and 28 vaccination cohort) between doses. Within each cohort, the first 36 participants (block 1) were randomly assigned to either the low dose vaccine or placebo, and then after 7 days of follow-up for safety after the first dose, another 36 (block 2) were randomly assigned to either high-dose vaccine or placebo. Phase 2 was initiated after all participants in phase 1 has finished a 7-day safety observation period after the first dose. As in phase 1, participants were recruited and allocated (1:1) with no specific randomisation to one of the two vaccination-schedule cohorts, and then randomly assigned within each cohort to either low-dose vaccine, high-dose vaccine, or placebo.

Participants were eligible if they were healthy and aged 18–59 years. The key exclusion criteria were high-risk epidemiology history within 14 days before enrolment (eg, travel or residence history in Wuhan city and surrounding areas or other communities with case reports; contact history with someone infected with SARS-CoV-2); SARS-CoV-2 specific IgG or IgM positive in serum; positive PCR test for SARS-CoV-2 from a pharyngeal or anal swab sample; axillary temperature of more than 37·0°C; and known allergy to any vaccine component. A complete list of exclusion criteria is in the protocol.

Written informed consent was obtained from each participant before enrolment. The clinical trial protocol and informed consent form were approved by the Jiangsu Ethics Committee (JSJK2020-A021–02). This study was conducted in accordance with the requirements of Good Clinical Practice of China and the International Conference on Harmonisation.

Randomisation and masking

In both phase 1 and 2, no specific randomisation was used when allocating participants to the vaccinations schedule cohorts. In phase 1, participants in blocks 1 and 2 in each schedule cohort were randomly assigned (2:1) to either CoronaVac or placebo, and in phase 2, participants in each schedule cohort were randomly assigned (2:2:1) to either low-dose CoronaVac, high-dose CoronaVac, or placebo. The randomisation codes for each vaccination schedule cohort were generated individually, using block randomisation with a block size of six in phase 1 and a block size of five in phase 2, using SAS software (version 9.4). The randomisation code was assigned to each participant in sequence in the order of enrolment, and then the participants received the investigational products labelled with the same code. The vaccine and the placebo are identical in appearance. All participants, investigators, and laboratory staff were masked to treatment allocation.

Procedures

The phase 1 clinical trial was run in a dose-escalation manner. First, participants in block 1 were given the low dose of vaccine, and only after a successful safety observation 7 days after the first dose was the trial able to proceed and participants in block 2 be given the high dose of vaccine. The criteria that had to be met from the 7-day safety observation were that no life-threatening adverse events occur, no more than 15% of vaccinated participants report severe adverse events, and no other safety concerns in the opinion of the data monitoring committee (DMC) occur. The same conditions needed to be met 7 days after the first dose in block 2 of the phase 1 trial before the study could proceed to the phase 2 trial.

CoronaVac is an inactivated vaccine candidate against COVID-19, created from African green monkey kidney cells (Vero cells) that have been inoculated with SARS-CoV-2 (CN02 strain). At the end of the incubation period, the virus was harvested, inactivated with β-propiolactone, concentrated, purified, and finally absorbed onto aluminium hydroxide. The aluminium hydroxide complex was then diluted in a sodium chloride, phosphate-buffered saline, and water solution before being sterilised and filtered ready for injection. The placebo is just the aluminium hydroxide diluent solution with no virus. Both the vaccine and placebo were prepared in a Good Manufacturing Practice-accredited facility of Sinovac Life Sciences (Beijing, China) that is periodically inspected by the Chinese National Medical Products Administration committee for compliance. Vaccine of 3 μg and 6 μg in 0·5 mL of aluminium hydroxide diluent per dose and placebo in ready-to-use syringes were administered intramuscularly according to the dosing schedule of either day 0 and day 14, or day 0 and day 28, depending on the cohort. These vaccine doses had been found to be sufficient for protection against SARS-CoV-2 challenge in macaques.[15] Cultivation technology by cell factory system (CellSTACK Cell Culture Chamber 10, Corning, Wujiang, China) was used in the preparation of the vaccine used in the phase 1 trial. However, for the phase 2 trial, we used a highly automated bioreactor (ReadyToProcess WAVE 25, GE, Umea, Sweden) to produce the vaccine to increase vaccine production capacity. After the immunogenicity results of the trial were obtained, we discovered that the change in manufacture of the vaccine optimised the cell culture and resulted in higher intact spike protein content of the vaccine batch for the phase 2 trial, which was unexpected. However, we were not aware of this antigen-level difference between the vaccine batches for the phase 1 and 2 trials when we obtained the ethical approval for the trials.

For the first 7 days after each dose, participants were required to record the injection-site adverse events (eg, pain, redness, swelling), or systemic adverse events (eg, allergic reaction, cough, fever) on paper diary cards. From day 8 to day 28 after each dose (and day 8 to day 14 for the first dose of the days 0 and 14 vaccination cohort), safety data were collected by spontaneous report from the participants combined with the regular visit (which occurred on day 8 and day 28 after each dose, and on

For the **protocol** see http://www.jscdc.cn/jkfw/kygz/202009/t20200930_69600.html

day 8 and day 14 for the first dose in the days 0 and 14 vaccination schedule cohort). Serious adverse events were collected through the trial and will be collected until 6 months after the last dose. The reported adverse events were graded according to the China National Medical Products Administration guidelines.[16] The causal association between adverse events and vaccination was determined by the investigators.

In the phase 1 trial, blood and urine samples were taken on day 3 after each dose and tested to investigate any abnormal changes of the haematology and biochemistry indexes. 7 days after each dose, blood and urine samples were taken to measure serum inflammatory factors including IL-2, IL-6, and TNF-α using the solid phase sandwich ELISA method to explore the underlying pathological immune responses. Blood samples were collected at days 0 (baseline), 7, 14, 21, 28, and 42 from participants in the day 0 and 14 vaccination cohort, and days 0, 28, 35, 42, and 56 from participants in the days 0 and 28 vaccination schedule cohort, to determine the levels of neutralising antibodies, receptor-binding domain (RBD)-specific IgG, S-specific IgG, and IgM. Additionally, T-cell responses were determined via IFN-γ detection on day 14 after each dose.

In the phase 2 trial, blood samples were collected on day 0, 28, and 56 from participants in the days 0 and 14 cohort, and on day 56 from participants in the days 0 and 28 cohort, to determine the levels of neutralising antibodies and RBD-specific IgG.

The neutralising antibodies to live SARS-CoV-2 (virus strain SARS-CoV-2/human/CHN/CN1/2020, GenBank number MT407649.1) were quantified using a micro cytopathogenic effect assay[17] with a minimum four-fold dilution, and neutralising antibodies to pseudovirus[18] were quantified with a minimum ten-fold dilution. The S-specific IgG and IgM were detected using the chemiluminescence qualitative kit (Auto Biotechnology, Zhengzhou, China). These antibody detection tests were done by the National Institute for Food and Drug Control (Beijing, China).

Additionally, antibody titres for RBD-specific IgG were quantified using the in-house ELISA kit from Sinovac, with a minimum 160-fold dilution. T-cell response was determined with the ELISpot method using a commercial kit (Human IFN γ ELISpotPRO [3420-2AST-10, AID]; Mabtech, Stockholm, Sweden). Further information on all methods is in the appendix 2 (pp 1–3). Additionally, in a post-hoc analysis, we tested serum samples from 117 convalescent patients who had previously had COVID-19 collected in the hospitals for neutralising antibodies to live SARS-CoV-2 using the same method as for the detection of serum neutralising antibodies to live SARS-CoV-2 in the phase 1 and 2 trials, to give a comparison of the vaccine-induced and infection-induced humoral immunity. Written informed consent was obtained from all these convalescent patients.

See Online for appendix 2

Outcomes

The primary safety endpoint was any adverse reactions within 28 days after each dose of study drug. Secondary safety endpoints were any abnormal changes in laboratory measurements at day 3 and in serum inflammatory factors 7 days after each dose of study drug. The secondary safety endpoints were prespecified only in the phase 1 trial.

The primary immunogenic endpoint was the seroconversion of neutralising antibodies to live SARS-CoV-2 at day 14 after the last dose in the days 0 and 14 vaccination cohort, or day 28 after the last dose in the days 0 and 28 vaccination cohort. Secondary immunogenic endpoints were geometric mean titres (GMTs) of neutralising antibodies to live SARS-CoV-2, RBD-specific IgG, S-specific IgG, and IgM. Exploratory endpoints were T-cell responses and, post hoc, GMTs of neutralising antibodies to psuedovirus. Seroconversion of antibodies was defined as a change from seronegative at baseline to seropositive or a four-fold titre increase if the participant was seropositive at baseline. The positive cutoff of the neutralising antibodies to live SARS-CoV-2 was 1/8, neutralising antibodies to pseudovirus was 1/30, and RBD-specific IgG was 1/160. Regarding the ELISpot measured T-cell response, the results were expressed as the number of spot-forming cells (SFCs) per 100 000 cells.

Other secondary endpoints are listed in the appendix 2 (p 4), including 6 month outcomes that are not available yet, which will be reported elsewhere.

Statistical analysis

We assessed the safety endpoints in the safety population, which included all participants who received at least one dose of study drug. We assessed immunogenic endpoints in the per-protocol population, which included all participants who completed their assigned two-dose vaccination schedule and with available antibody results.

We did not determine the sample size on the basis of a statistical power calculation, but followed the requirement of the National Medical Products Administration in China—ie, recruitment of at least of 20–30 participants in phase 1 and 500 participants in phase 2.

We used the Pearson χ^2 test or Fisher's exact test for the analysis of categorical outcomes. We calculated 95% CIs for all categorical outcomes using the Clopper-Pearson method. We calculated GMTs and corresponding 95% CIs on the basis of standard normal distribution of the log-transformed antibody titre. We used the ANOVA method to compare the log-transformed antibody titre. When the comparison among all three groups showed significant difference, we then did pairwise comparisons. Hypothesis testing was two-sided and we considered p values of less than 0·05 to be significant.

An independent data monitoring committee consisted of one independent statistician, one clinician, and one epidemiologist was established before commencement of the study. Safety data were assessed and

reviewed by the committee to ensure the suspension criteria of the dose-escalation part of phase 1 were not met and allow the further proceeding of the clinical trial.

We used SAS (version 9.3) for all analyses. This trial is registered with ClinicalTrials.gov, NCT04352608.

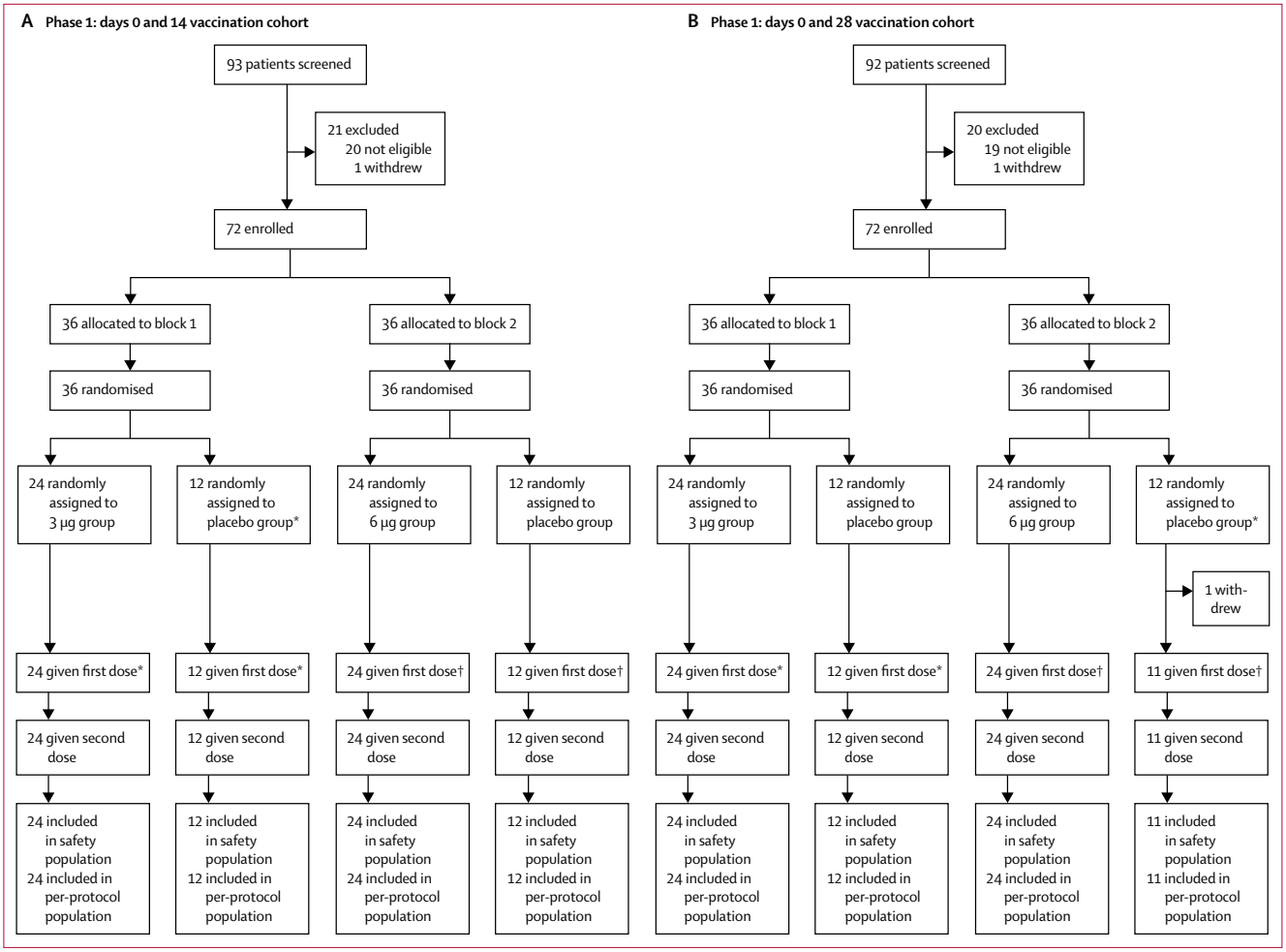
Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All the authors have full access to all the data in the study and the corresponding authors had final responsibility for the decision to submit for publication.

Results

Between April 16 and April 25, 2020, 185 individuals were screened and 144 participants were enrolled in the phase 1 trial, and between May 3 and May 5, 2020, 662 individuals were screened and 600 participants were enrolled in the phase 2 trial. 743 participants received at least one dose of the investigational product (143 for phase 1 and 600 for phase 2) and were included in the safety population (figure 1). 143 participants in phase 1 and 591 participants in phase 2 were eligible for the immunogenic evaluation (per-protocol population; figure 1). Baseline demographic characteristics of the participants in the safety population at enrolment were similar among the treatment groups in terms of sex, nationality, and mean age (table 1).

In the phase 1 trial, the overall incidence of adverse reactions was seven (29%) of 24 participants in the 3 μg group, nine (38%) of 24 in the 6 μg group, and two (8%) of 24 in the placebo group in the days 0 and 14 vaccination cohort; and three (13%) of 24 in the 3 μg group, four (17%) of 24 in the 6 μg group, and three (13%) of 23 in the placebo group in the days 0 and 28 vaccination



(Figure 1 continues on next page)

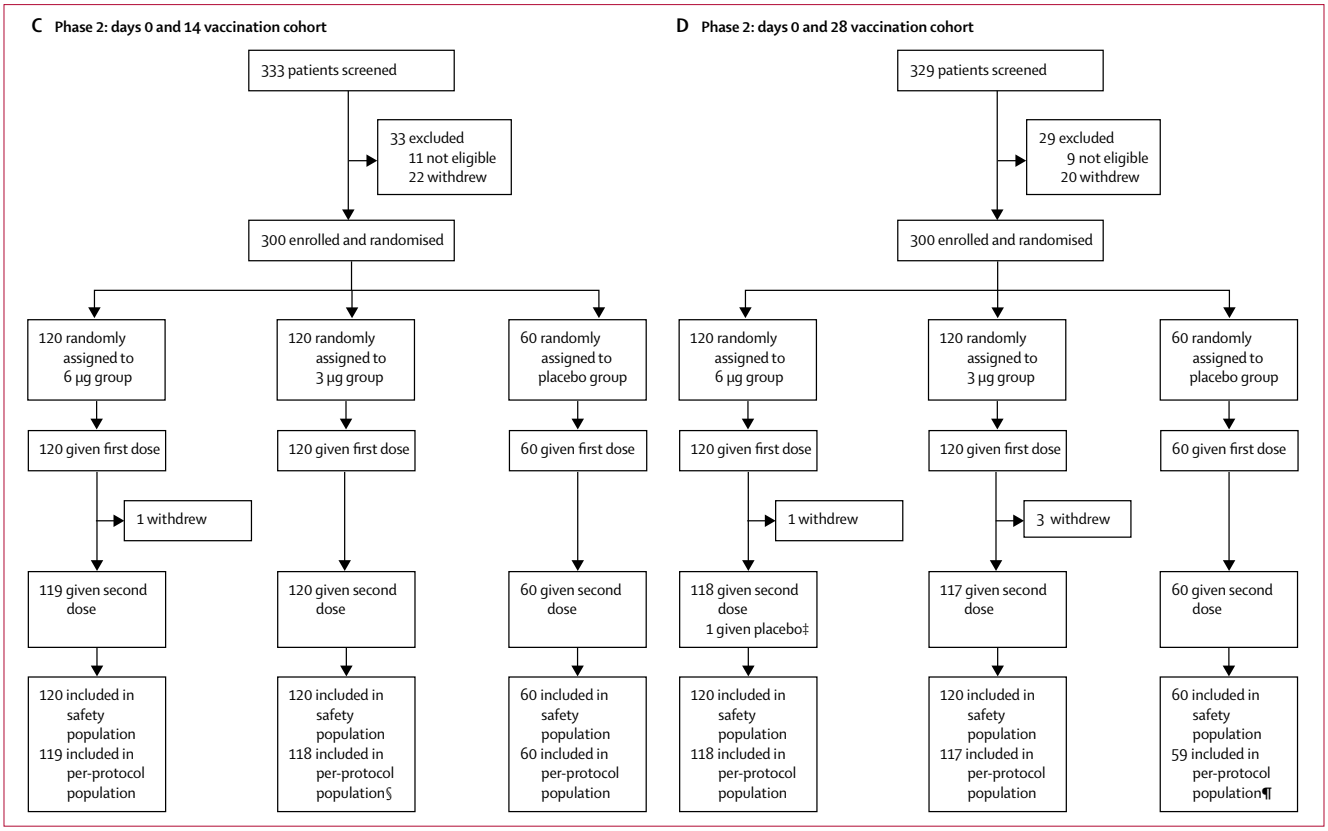


Figure 1: **Study profile**

*7 days after first dose, safety observation was done, and safety criteria were met, as determined by the data monitoring committee, participants in block 2 were then given their first dose of vaccine. †7 days after first dose of study drug in block 2, if safety criteria were met as determined by the data monitoring committee, participants enrolled in phase 2 were started on study treatment. ‡A participant in the 6 μg group was mistakenly given placebo rather than vaccine at the second dose; therefore, this participant was included in the 6 μg group dataset in the overall safety evaluation but not in the immunogenicity analysis. §Two participants did not have available antibody results, and so were not included in the immunogenicity analysis. ¶One participant did not have available antibody results, and so was not included in the immunogenicity analysis.

	3 μg group	6 μg group	Placebo group	Overall
Days 0 and 14 vaccination cohorts, pooled				
Participants	144	144	84	372
Sex				
Female	77 (53%)	86 (60%)	44 (52%)	207 (56%)
Male	67 (47%)	58 (40%)	40 (48%)	165 (44%)
Han nationality	144 (100%)	144 (100%)	84 (100%)	372 (100%)
Age, years	42·4 (10·2)	42·8 (9·0)	42·4 (8·8)	42·6 (9·4)
Days 0 and 28 vaccination cohorts, pooled				
Participants	144	144	83	371
Sex				
Female	75 (52%)	70 (49%)	45 (54%)	190 (51%)
Male	69 (48%)	74 (51%)	38 (46%)	181 (49%)
Han nationality	144 (100%)	144 (100%)	83 (100%)	371 (100%)
Age, years	41·8 (9·4)	41·2 (10·2)	44·1 (9·1)	42·1 (9·7)

Data are n, n (%), or mean (SD).

Table 1: **Baseline demographic characteristics for the safety population, phases 1 and 2 combined**

cohort, with no significant difference seen among the three groups for both vaccination schedules (figure 2; appendix 2 pp 5–6). The most common symptom was injection-site pain, which was reported by four (17%) participants in the 3 μg group, five (21%) in the 6 μg, and one (4%) in the placebo group in the days 0 and 14 vaccination cohort and three (13%) in the 3 μg group, three (13%) in the 6 μg group, and three (13%) in the placebo group in the days 0 and 28 vaccination cohort. Most adverse reactions were mild (grade 1) in severity and participants recovered within 48 h. Only one case of acute hypersensitivity with manifestation of urticaria 48 h after the first dose of study drug was reported in the 6 μg group (one [4%] of 24) in the days 0 and 14 vaccination cohort, which was graded as severe and considered to be possibly related to vaccination. The participant was given chlorphenamine and dexamethasone and recovered within 3 days, and no similar reaction was observed after the second dose of vaccine. No vaccine-related serious adverse events were noted within 28 days of vaccination (figure 2; appendix 2 pp 4–5).

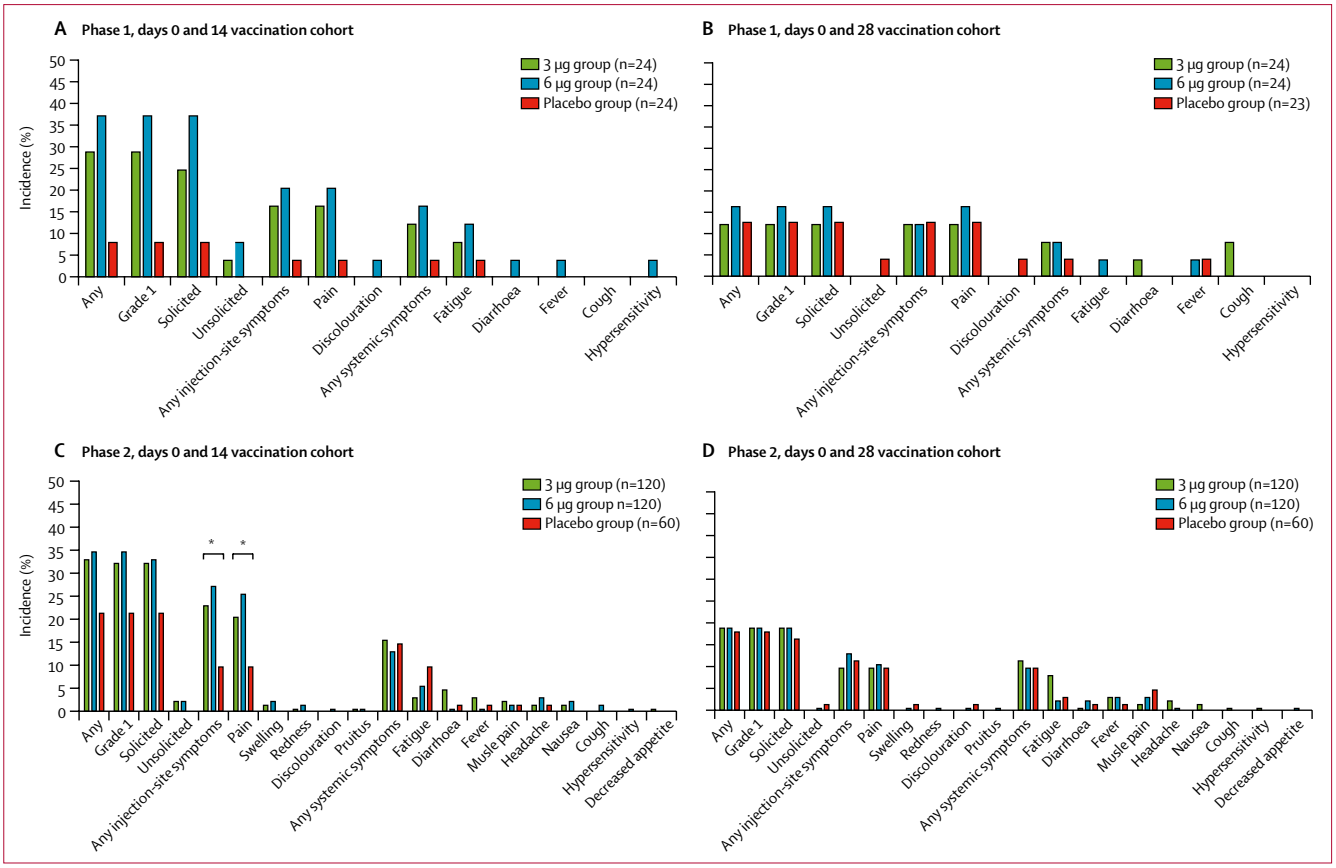


Figure 2: **Incidence of adverse reactions reported within 28 days after second dose of study drug, in the days 0 and 14 vaccination cohort in phase 1 (A) and phase 2 (C) and in the days 0 and 28 vaccination cohort in phase 1 (B) and phase 2 (D)**

Adverse reactions refer to the adverse events related to the vaccination. Rare injection-site symptoms reported only in the days 0 and 14 vaccination cohort are not shown in the figure and are listed in appendix 2 along with all adverse reactions after the first and second dose (pp 4–13). *The p value of comparison among three groups is significant for the incidence of any injection-site symptoms (p=0·02) and injection-site pain (p=0·04).

Additionally, ten (7%) of 143 participants in phase 1 had a clinically significant increase of laboratory indicators at day 3 after vaccination (appendix 2 pp 15–16), but none was considered to be related to the vaccination. No significant increases in inflammatory factors in serum were detected at day 7 after each dose (appendix 2 pp 17–18).

At baseline, none of the participants in the phase 1 trial had any detectable neutralising antibodies to live SARS-CoV-2. The seroconversion rates of neutralising antibodies were 11 (46%) of 24 participants in the 3 μg group (GMT 5·6 [95% CI 3·6–8·7]) versus 12 (50%) of 24 participants in the 6 μg group (7·7 [5·2–11·5]) versus none of 24 participants in the placebo group (2·0 [2·0–2·0]) at 14 days after the second dose, and six (25%) participants in the 3 μg group (5·4 [3·6–8·1] versus 20 (83%) in the 6 μg group (15·2 [11·2–20·7]) versus none in the placebo group (2·0 [2·0–2·0]) at 28 days after the second dose in the days 0 and 14 vaccination cohort; and 19 (79%) of 24 participants in the 3 μg group (16·0 [10·4–24·7]) versus 20 (83%) of 24 in the 6 μg group (25·9 [14·6–46·1) versus none of 23 in the placebo group (2·0 [2·0–2·0]) at 14 days after the second dose, and 20 (83%) in the 3 μg group (19·0 [13·2–27·4] versus 19 (79%) in the 6 μg group (29·6 [17·9–48·9]) versus one (4%) in the placebo group (2·2 [1·8–2·8]) at 28 days after the second dose in the days 0 and 28 vaccination cohort (table 2, figure 3; appendix 2 p 19). The seroconversion rates of RBD-specific IgG were 20 (83%) of 24 participants in the 3 μg group (GMT 465·8 [95% CI 277·6–781·7] versus 24 (100%) of 24 participants in the 6 μg group (987·0 [647·8–1504·0]) versus two (8%) of 24 participants in the placebo group (84·8 [78·0–92·1]) at 14 days after the second dose, and 21 (88%) in the 3 μg group (465·8 [288·1–753·1]) versus 24 (100%) in the 6 μg group (1395·9 [955·2–2039·7]) versus two (8%) in the placebo group (89·8 [76·1–105·9]) at 28 days after the second dose in the days 0 and 14 vaccination cohort; and 24 (100%) of 24 participants in the 3 μg group (1365·1 [881·4–2086·4]) versus 24 (100%) of 24 participants in the 6 μg group (2152·7 [1446·1–3204·6])

	3 μg group	6 μg group	Placebo group	p value*
Phase 1				
Days 0 and 14 vaccination cohort				
Neutralising antibodies to live SARS-CoV-2				
Day 14	11/24 (45·8%; 25·6–67·2)	12/24 (50·0%; 29·1–70·9)	0/24 (0·0%; 0·0–14·3)	0·77
Day 28	6/24 (25·0%; 9·8–46·7)	20/24 (83·3%; 62·6–95·3)	0/24 (0·0%; 0·0–14·3)	<0·0001
RBD-IgG				
Day 14	20/24 (83·3%; 62·6–95·3)	24/24 (100%; 85·8–100)	2/24 (8·3%; 1·0–27·0)	0·11
Day 28	21/24 (87·5%; 67·6–97·3)	24/24 (100%; 85·8–100)	2/24 (8·3%; 1·0–27·0)	0·23
Days 0 and 28 vaccination cohort				
Neutralising antibodies to live SARS-CoV-2				
Day 14	19/24 (79·2%; 57·9–92·9)	20/24 (83·3%; 62·6–95·3)	0/23 (0·0%; 0·0–14·8)	1·00
Day 28	20/24 (83·3%; 62·6–95·3)	19/24 (79·2%; 57·9–92·9)	1/23 (4·4%; 0·1–22·0)	1·00
RBD-IgG				
Day 14	24/24 (100%; 85·8–100)	24/24 (100%; 85·8–100)	0/23 (0·0%; 0·0–14·8)	1·00
Day 28	24/24 (100%; 85·8–100)	24/24 (100%; 85·8–100)	0/23 (0·0%; 0·0–14·8)	1·00
Phase 2				
Days 0 and 14 vaccination cohort				
Neutralising antibodies to live SARS-CoV-2				
Day 14	109/118 (92·4%; 86·0–96·5)	117/119 (98·3%; 94·1–99·8)	2/60 (3·3%; 0·4–11·5)	0·030
Day 28	111/118 (94·1%; 88·2–97·6)	117/118 (99·2%; 95·4–100)	0/60 (0·0%; 0·0–6·0)	0·066
RBD-IgG				
Day 14	111/115 (96·5%; 91·3–99·0)	118/118 (100%; 96·9–100)	0/56 (0·0%; 0·0–6·4)	0·058
Day 28	111/114 (97·4%; 92·5–99·5)	118/118 (100%; 96·9–100)	0/57 (0·0%; 0·0–6·3)	0·12
Days 0 and 28 vaccination cohort				
Neutralising antibodies to live SARS-CoV-2				
Day 28	114/117 (97·4%; 92·7–99·5)	118/118 (100%; 96·9–100)	0/59 (0·0%; 0·0–6·1)	0·12
RBD-IgG				
Day 28	116/117 (99·2%; 95·3–100)	117/117 (100%; 96·9–100)	4/59 (6·8%; 1·9–16·5)	1·00

Data are n/N (%; 95% CI). Timepoints refer to the number of days since the second dose of vaccine in the schedule. RBD=receptor binding domain. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. *p values are for comparisons between the 3 μg and 6 μg groups.

Table 2: **Seroconversion rates of neutralising antibodies to live SARS-CoV-2 and RBD-specific IgG**

versus none of 23 participants (80·0 [80·0-80·0]) in the placebo group at 14 days after the second dose, and 24 (100%) in the 3 μg group (1045·7 [721·6–1515·5]), versus 24 (100%) in the 6 μg group (1917·9 [1344·8–2735·2]) versus none in the placebo group (80·0 [80·0–80·0]) 28 days after the second dose in the days 0 and 28 vaccination cohort (table 2, figure 3; appendix 2 p 19). The dynamic changes of RBD-specific IgG, S-specific IgG, S-specific IgM, and neutralising antibodies to pseudovirus are shown in the appendix 2 (pp 19–23), showing that the antibody levels did not significantly increase until after the second dose of vaccine.

At 14 days after the second dose of study drug, the average IFN-γ-positive SFCs per 100 000 cells were 7·4 (95% CI 3·9 to 11·1) in the 3 μg group, 3·9 (1·0 to 6·7) in the 6 μg group, and 1·5 (0·2 to 2·9) in the placebo group for the days 0 and 14 vaccination cohort; and 3·4 (0·9 to 5·7) in the 3 μg group, 1·2 (0·5 to 1·8) in the 6 μg group, and 1·2 (–0·1 to 2·5) in the placebo group for the days 0 and 28 vaccination cohort (appendix 2 pp 25–26).

In the phase 2 trial, the overall incidence of adverse reactions were 40 (33%) of 120 in the 3 μg group, 42 (35%) of 120 in the 6 μg group, and 13 (22%) of 60 in the placebo group for the days 0 and 14 vaccination cohort and 23 (19%) of 120 in the 3 μg group, 23 (19%) of 120 in the 6 μg group, and 11 (18%) of 60 in placebo group in the days 0 and 28 vaccination cohort, with no significant difference between the three groups for both schedules. However, the p value of comparison among the three groups was significant for the incidence of any injection-site symptoms (p=0·02) and injection-site pain (p=0·04; figure 2; appendix 2 pp 7–10). The most common symptom was injection-site pain, which occurred in 25 (21%) of 120 participants in the 3 μg group, 31 (26%) of 120 in the 6 μg group, and six (10%) of 60 in the placebo group for the days 0 and 14 vaccination cohort, and 12 (10%) of 120 in the 3 μg group, 13 (11%) of 120 in the 6 μg group, and six (10%) of 60 in the placebo group in the days 0 and 28 vaccination cohort. Most adverse reactions were mild (grade 1) in severity and the participants recovered within 48 h. No vaccine-related serious adverse events were noted within 28 days of the second dose of vaccine (figure 2; appendix 2 pp 7–10)

In the phase 2 trial, at baseline, none of the participants had any detectable neutralising antibodies. The seroconversion rates of neutralising antibodies to live SARS-CoV-2 were 109 (92%) of 118 participants in the 3 μg group (GMT 27·6 [95% CI 22·7–33·5]) versus 117 (98%) of 119 participants in the 6 μg group (34·5 [28·5–41·8] versus two (3%) of 60 participants in the placebo group (2·3 [2·0–2·5]) at 14 days after the second dose, and 111 (94%) of 118 in the 3 μg group (23·8 [20·5–27·7]) versus 117 (99%) of 118 in the 6 μg group (30·1 [26·1–34·7]) versus none of 60 in the placebo group (2·0 [2·0–2·0]) at 28 days after the second dose in the day 0 and 14 vaccination cohort; and 114 (97%) of 117 participants in the 3 μg group (44·1 [37·2–52·2]) versus 118 (100%) of 118 participants in the 6 μg group (65·4 [56·4–75·9]) versus none of 59 participants in the placebo group (2·0 [2·0–2·1]) at 28 days after the second dose in the days 0 and 28 vaccination cohort (table 2, figure 3). In post-hoc analyses, the neutralising antibody titres after the second dose of vaccine was lower in all participants who received the vaccine than was detected in 117 convalescent asymptomatic patients who had previously had COVID-19

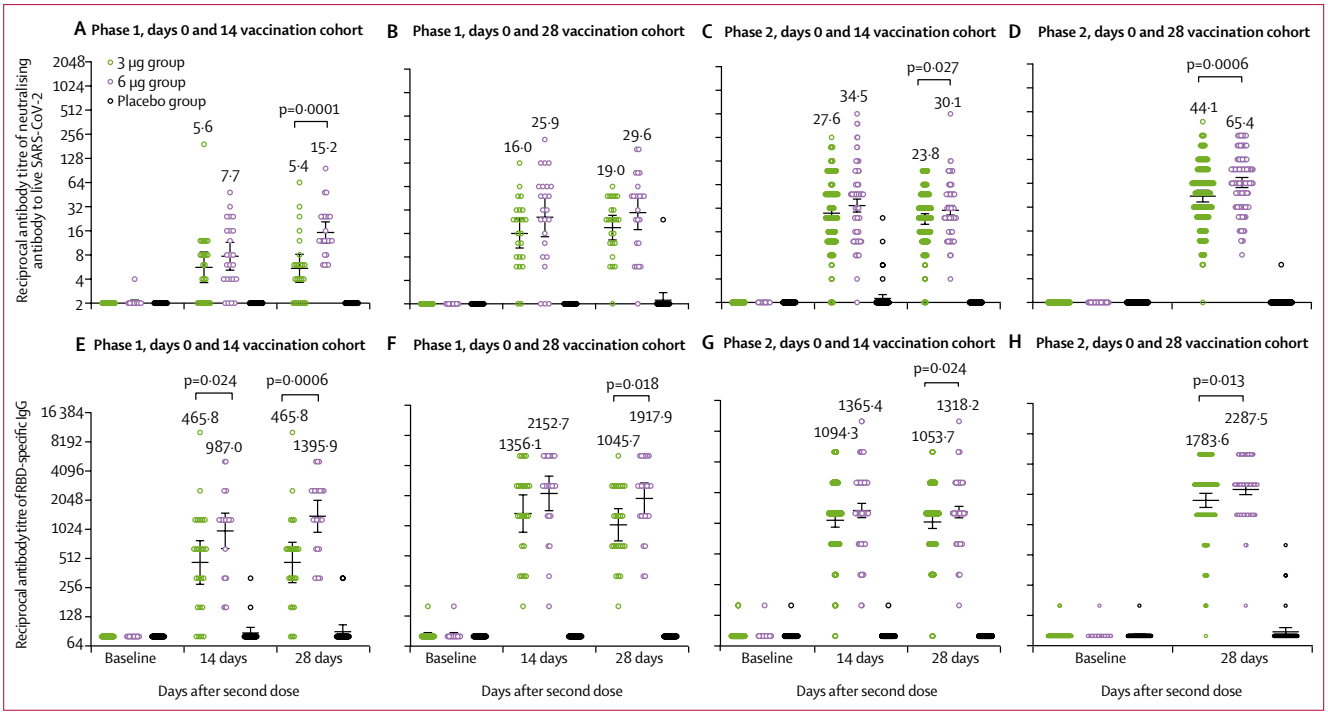


Figure 3: **Antibody titres of neutralising antibodies to live SARS-CoV-2 (A–D) and RBD-specific IgG (E–H) induced after two doses of CoronaVac or placebo given in the days 0 and 14 and days 0 and 28 vaccination cohorts, in the phase 1 and phase 2 trials**

The error bars indicate the 95% CI of the GMT and the spots indicated the individual antibody titres, with the numbers above the spots showing the GMT estimate. Only p values for significant differences are shown on the figure, all p values for all data are in appendix 2 (p 19). GMT=geometric mean titre. RBD=receptor binding domain. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

(GMT 163·7 [95% CI 128·5–208·6]; table 2, figure 3; appendix 2 p 24). The seroconversion rates of RBD-specific IgG were 111 (97%) of 115 participants in the 3 μg group (GMT 1094·3 [95% CI 936·7–1278·4]) versus 118 (100%) of 118 participants in the 6 μg group (1365·4 [1160·4–1606·7]) versus none of 56 participants in the placebo group (81·0 [79·0–83·0]) at 14 days after the second dose and 111 (97%) of 114 in the 3 μg group (1053·7 [911·7–1217·7]) versus 118 (100%) of 118 in the 6 μg group (1318·2 [1156·9–1501·9]) versus none of 57 in the placebo group (80·0 [80·0–80·0]) at 28 days after the second dose in the day 0 and 14 vaccination cohort; and 116 (99%) of 117 in the 3 μg group (1783·6 [1519·3–2093·8]) versus 117 (100%) of 117 in the 6 μg group (2287·5 [2038·2–2567·3]) versus four (7%) of 59 in the placebo group (87·9 [79·7–96·9]) at 28 days after the second dose in the days 0 and 28 vaccination cohort (table 2, figure 3).

Based on the pooled data of the phase 1 and 2 trials (two vaccination cohorts pooled), the correlation coefficient between the neutralising antibody to live SARS-CoV-2 and RBD-specific IgG was 0·85 (95% CI 0·82–0·92) using the antibody titre at 28 days after the second dose of vaccine, and was 0·80 (0·75–0·86) using the titre 14 days after the second. The correlation coefficient between the neutralising antibody to live SARS-CoV-2 and the neutralising antibody to pseudovirus was 0·82 (0·76–0·88) using the antibody titre at 14 days after the second dose (no data taken at day 28). The correlation coefficient between the neutralising antibody to pseudovirus and RBD-specific IgG was 0·73 (0·66–0·80) using the antibody titre at 14 days after the second dose (no data taken at day 28; appendix 2 p 24).

Discussion

We found that two doses of CoronaVac at different concentrations and using different dosing schedules were well tolerated and moderately immunogenic in healthy adults aged 18–59 years. The incidence of adverse reactions in the 3 μg and 6 μg group were similar, indicating no dose-related safety concerns but more long-term follow-up is needed. Furthermore, most adverse reactions were mild, with the most common symptom being injection-site pain, which is in accordance with previous findings for another inactivated COVID-19 vaccine from Sinopharm (Beijing China).[14] Compared with other COVID-19 vaccine candidates, such as viral-vectored vaccines or DNA or RNA vaccines, the occurrence of fever after vaccination with CoronaVac was relatively low.[10,11,13]

Over the course of the phase 1/2 trial, we changed the production process of the vaccine from the use of a cell factory process (which was used in our preclinical and

phase 1 study to generate a 50 L culture of Vero cells) to use of a bioreactor for phase 2. The bioreactor process enabled use to optimise the process for growing cells, with precise control over cell culture parameters like dissolved oxygen, pH, and carbon dioxide and oxygen gas levels. We made this change to increase vaccine production capacity and meet biosafety requirements. Pre-clinical data for each phase trial (data not shown) indicated that the safety profiles of vaccines prepared via the new bioreactor process and old process are similar. Notably, immune responses in phase 2 were much better than those recorded in phase 1, with seroconversion rates over 90% in both the 3 μg and 6 μg groups. To investigate the reason for this change, we did a protein composition analysis of the purified inactivated SARS-CoV-2 virions and found that the bioreactor-produced vaccine had a higher redundancy of intact spike protein (molecular mass approximately 180 kDa) than did the vaccine produced via the cell factory process (appendix 2 p 27). Quantitative analysis showed that the intact spike protein accounted for approximately 3·7% of total protein mass of the vaccine used in phase 1 and approximately 7·0% of total protein mass of the vaccine used in phase 2 trials. Electron microscopic examination of the samples further verified that the average number of spikes per virion of the viral sample used in the phase 2 trial was almost double the number of spikes per virion of the sample used in phase 1 trial (appendix 2 p 27). These observations highlight the importance of developing an optimum manufacturing process and the integration of multi-disciplinary techniques, such as genomics and structural biology to support a new era of precision vaccinology.

The immune response induced by 3 μg and 6 μg of vaccine in 0·5 mL of diluent per dose was similar in this study. As anticipated, after two doses of vaccine, immune responses induced by the days 0 and 28 vaccination schedule were larger than those induced by the days 0 and 14 vaccination schedule, regardless of the dose. However, quick antibody responses could be induced within a relatively short time by using a day 0 and 14 vaccination schedule, which might be suitable for emergency use and is of vital importance during the COVID-19 pandemic. Regarding the days 0 and 28 vaccination schedule, a more robust antibody response was generated and longer persistence could be expected than with the days 0 and 14 schedule, which supports potential routine use of the vaccine according to this schedule when the epidemic risk of COVID-19 is low. However, the actual immune persistence of the two schedules needs to be verified in future studies.

In the phase 2 trial, the level of neutralising antibodies included by the vaccine at day 28 after the last dose of vaccine ranged from a GMT of 23·8 to 65·4, depending on the vaccination schedule, which was lower than those of convalescent patients who previously had COVID-19 with an average GMT level of 163·7, tested by the same method in the same laboratory.[19] However, we still think that CoronaVac could provide satisfying protection against COVID-19 on the basis of the following three reasons. First, from the experiences of other vaccines, such as the enterovirus 71 and varicella vaccines, most of the surrogate endpoints based on neutralising antibody titres have ranged from 8 to 24.[20,21] Second, our preclinical study[15] indicated that the neutralising antibody titres of 1/24 elicited in macaque models conferred complete protection against SARS-CoV-2. Third, although several studies have found that antibody responses generated from natural infection with coronaviruses (eg, SARS-CoV-2, severe acute respiratory syndrome coronavirus, and Middle East respiratory syndrome coronavirus) might decrease substantially over time,[22–24] reinfection in these patients has rarely been reported,[25–27] which indicates that immunological memory might have an important role of prevention of re-infections. Therefore, the antibody level itself might not be the key for a successful COVID-19 vaccine, but rather the establishment of a recallable specific immune response to SARS-CoV-2. Furthermore, the efficacy of the investigational vaccine and its surrogate endpoint need to be determined in a future phase 3 trial. Additionally, comparability of our serum antibody results with those of other COVID-19 vaccine studies is restricted.

Two participants in the placebo group in the phase 1 trial and four in the placebo group in the phase 2 trial had seroconversion of anti-RBD IgG after vaccination, and one participant given placebo in the phase 1 trial and two in the phase 2 trial had seroconversion of neutralising antibodies after vaccination.

CoronaVac was well tolerated and induced humoral responses against SARS-CoV-2, which supported the approval of emergency use of CoronaVac in China, and three phase 3 clinical trials that are ongoing in Brazil (NCT04456595), Indonesia (NCT04508075), and Turkey (NCT04582344). Taking safety, immunogenicity, and production capacity into account, the low dose of 3 μg of CoronaVac in 0·5 mL of diluent, with a day 0 and 14 vaccination schedule, is being investigated in these ongoing trials. And the days 0 and 28 vaccination schedule with 3 μg of Coronavac in 0·5 mL of diluent will also be investigated in future phase 3 clinical trials. The protective efficacy of CoronaVac remains to be determined.

Our study had several limitations. First, we did not assess the T cell responses in the phase 2 trial; however, the response of type 1 T-helper cells and type 2 T-helper cells induced by CoronaVac will be studied in the ongoing phase 3 study in Brazil (NCT04456595). Second, we only reported immune response data for healthy adults, and did not include individuals from more susceptible groups in our study population (eg, older individuals [aged ≥60 years] or with comorbidities); and data on immune persistence is not yet available, which need to be further studied. Third, the calculated p values presented in this study cannot support any powerful statistical conclusions, and are only for reference and so

should be interpreted with caution. Additionally, the T-cell responses measured by ELISpot were low in participants who were given vaccine, which provided no clear evidence that the vaccine induced T-cell responses. The assessment of immune reactions mediated by CD8 cells was not included in our study design, because inactivated vaccines are not thought to induce CD8 T-cell responses. Finally, the change in the manufacturing of vaccine batches for the phase 2 trial resulted in a higher level of the spike antigen contained in the vaccine than was used in the phase 1 trial. Although the change in manufacturing process was planned, the difference in antigenicity of the vaccines was not anticipated, and could potentially bring additional risks for the recipients of the vaccine. Fortunately, the safety profiles of the vaccines in the phase 1 and 2 trials were similar, although the vaccines for the phase 2 trial had substantially stronger immunogenicity than did the vaccines for phase 1 trial. However, the comparisons between the vaccine batches were also not an a-priori defined outcome or sufficiently powered.

In summary, CoronaVac was well tolerated and induced humoral responses against SARS-CoV-2, which suppored the approval of emergency use of CoronaVac in China and in three phase 3 studies. The protective efficacy of CoronaVac remains to be determined.

Contributors

YZ, GZ, HP, and CL were co-first authors of this manuscript. FZ was the principal investigator and HP was the coprincipal investigator of this trial. FZ, GZ, RT, and QG designed the trial and study protocol. YZ, YaH, and WH contributed to the literature search. All authors had access to data and GZ, FZ, and HP verified the data. WH, JL, XW wrote the first draft the manuscript. FZ, YZ, GZ, WY, YaH, and MY contributed to the data interpretation and revision of the manuscript. YuH monitored the trial. XC, XL, CJ, and YS were responsible for the site work including the recruitment, follow up, and data collection, and KC was the site coordinator. CL and ZC were responsible to the laboratory analysis.

Declaration of interests

QG is an employee of Sinovac Life Sciences. GZ, YaH, WH, WY, and YuH are employees of Sinovac Biotech. All other authors declare no competing interests.

Data sharing

The individual participant-level data that underlie the results reported in this Article will be shared after deidentification (text, tables, figures, and appendices). This clinical trial is ongoing, and all the individual participant data cannot be available until after the immune persistence assessments have been done. The data will be available immediately after publication and finalisation of the complete clinical study report for at least 6 months. Supporting clinical documents including study protocol, statistical analysis plan, and the informed consent form will be available immediately after publication of this Article for at least 1 year. Information on how to access the supporting clinical documents is available online. Researchers who provide a scientifically sound proposal will be allowed to access the de-identified individual participant data. Proposals should be sent to the corresponding authors, at jszfc@vip.sina.com or gaoq@sinovac.com. These proposals will be reviewed and approved by the sponsor, investigator, and collaborators on the basis of scientific merit. To gain access, data requestors will need to sign a data access agreement.

Acknowledgments

This study was funded by the National Key Research and Development Program (2020YFC0849600) and Beijing Science and Technology Program (Z201100005420023).

References

1 Walker PGT, Whittaker C, Watson OJ, et al. The impact of COVID-19 and strategies for mitigation and suppression in low- and middle-income countries. *Science* 2020; **369:** 413–22.

2 WHO. Coronavirus disease (COVID-19) situation report - 163. Geneva: World Health Organization, 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200701-covid-19-sitrep-163.pdf?sfvrsn=c202f05b_2 (accessed Nov 2, 2020).

3 Flaxman S, Mishra S, Gandy A, et al. Estimating the effects of non-pharmaceutical interventions on COVID-19 in Europe. *Nature* 2020; **584:** 257–61.

4 Sanche S, Lin YT, Xu C, Romero-Severson E, Hengartner N, Ke R. High contagiousness and rapid spread of severe acute respiratory syndrome coronavirus 2. *Emerg Infect Dis* 2020; **26:** 1470–77.

5 WHO. Draft landscape of COVID-19 candidate vaccines. Geneva: World Health Organization, Oct 19, 2020. https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines (accessed Oct 28, 2020).

6 Krammer F. SARS-CoV-2 vaccines in development. *Nature* 2020; **586:** 516–27.

7 Callaway E. The race for coronavirus vaccines: a graphical guide. *Nature* 2020; **580:** 576–77.

8 Corey L, Mascola JR, Fauci AS, Collins FS. A strategic approach to COVID-19 vaccine R&D. *Science* 2020; **368:** 948–50.

9 Graham BS. Rapid COVID-19 vaccine development. *Science* 2020; **368:** 945–46.

10 Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2 — preliminary report. *N Engl J Med* 2020; published July 14. https://doi.org/10.1056/NEJMoa2022483.

11 Zhu F-C, Li Y-H, Guan X-H, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. *Lancet* 2020; **395:** 1845–54.

12 Xia S, Zhang Y, Wang Y, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. *Lancet Infect Dis* 2020; published online Oct 15. https://doi.org/10.1016/S1473-3099(20)30831-8.

13 Folegatti PM, Ewer KJ, Aley PK, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* 2020; **396:** 467–78.

14 Xia S, Duan K, Zhang Y, et al. Effect of an inactivated vaccine against SARS-CoV-2 on safety and immunogenicity outcomes: interim analysis of 2 randomized clinical trials. *JAMA* 2020; **324:** 951–60.

15 Gao Q, Bao L, Mao H, Wang L, Qin C. Development of an inactivated vaccine candidate for SARS-CoV-2. *Science* 2020: **369:** 77–81.

16 Guidelines for grading standards of adverse events in clinical trials of preventive vaccines. China National Medical Products Administration, 2019. https://www.nmpa.gov.cn/xxgk/ggtg/qtggtg/20191231111901460.html (accessed Nov 3, 2020).

17 Li P, Liang Z, Gao Q, et al. Safety and immunogenicity of a novel human enterovirus 71 (EV71) vaccine: a randomized, placebo-controlled, double-blinded, phase I clinical trial. *Vaccine* 2012; **30:** 3295–303.

18 Nie J, Li Q, Wu J, et al. Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. *Emerg Microbes Infect* 2020; **9:** 680–86.

19 Wang X, Guo X, Xin, et al. Neutralizing antibody responses to severe acute respiratory syndrome coronavirus 2 in coronavirus disease 2019 inpatients and convalescent patients. *Clin Infect Dis* 2020; published online June 4. https://doi.org/10.1093/cid/ciaa721.

20 Hao B, Chen Z, Zeng G, et al. Efficacy, safety and immunogenicity of live attenuated varicella vaccine in healthy children in China: double-blind, randomized, placebo-controlled clinical trial. *Clin Microbiol Infect* 2019; **25:** 1026–31.

21 Jin P, Li J, Zhou Y, et al. Immunological surrogate endpoints to evaluate vaccine efficacy. *Chin J Prev Med* 2015; **49:** 1110–14 (in Chinese).

22 Chen W, Xu Z, Mu J, et al. Antibody response and viraemia during the course of severe acute respiratory syndrome (SARS)-associated coronavirus infection. *J Med Microbiol* 2004; **53:** 435–38.

For **information on how to access document** see http://www.jshealth.com/

23 Prévost J, Gasser R, Beaudoin-Bussières G, et al. Cross-sectional evaluation of humoral responses against SARS-CoV-2 spike. *Cell Rep Med* 2020; **1:** 100126.

24 Payne DC, Iblan I, Rha B, et al. Persistence of antibodies against Middle East respiratory syndrome coronavirus. *Emerg Infect Dis* 2016; **22:** 1824–26.

25 Zhang K, Lau JY-N, Yang L, Ma Z-G. SARS-CoV-2 reinfection in two patients who have recovered from COVID-19. *Precis Clin Med* 2020; published online Sept 4. https://doi.org/10.1093/pcmedi/pbaa031.

26 Chan PKS, Lui G, Hachim A, et al. Serologic responses in healthy adult with SARS-CoV-2 reinfection, Hong Kong, August 2020. *Emerg Infect Dis* 2020; published online Oct 22. https://doi.org/10.3201/eid2612.203833.

27 Huang AT, Garcia-Carreras B, Hitchings MDT, et al. A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nat Commun* 2020; **11:** 4704.

JAMA Insights

Reactogenicity Following Receipt of mRNA-Based COVID-19 Vaccines

Johanna Chapin-Bardales, PhD, MPH; Julianne Gee, MPH; Tanya Myers, PhD, MSc

In December 2020, 2 mRNA-based COVID-19 vaccines (Pfizer-BioNTech and Moderna) were granted Emergency Use Authorization by the US Food and Drug Administration as 2-dose series and recommended for use by the Advisory Committee on Immunization Practices.[1-3] In late February 2021, the US Food and Drug Administration granted Emergency Use Authorization for a third COVID-19 vaccine, a single-dose adenovirus vector-based vaccine from Janssen (Johnson & Johnson).

Supplemental content

In clinical trials of the mRNA-based 2-dose vaccines, participants reported local and systemic reactions (reactogenicity).[4,5] Frequently reported reactions included injection site pain, fatigue, and headache; greater reactogenicity was reported following the second dose.[4,5] Continued monitoring of reactogenicity of COVID-19 vaccines outside of clinical trial settings may provide additional information for health care practitioners and the public about transient local and systemic reactions following COVID-19 vaccination.

V-safe Active Surveillance System

To facilitate rapid assessment of COVID-19 vaccines, in 2020, the Centers for Disease Control and Prevention (CDC) established v-safe, a new active surveillance system for collecting near–real-time data from COVID-19 vaccine recipients in the US. V-safe participants voluntarily self-enroll and receive periodic smartphone text messages to initiate web-based health surveys from the day of vaccination (day 0) through 12 months after the final dose of a COVID-19 vaccine.[6] From day 0 through day 7 after each vaccine dose, participants are asked questions about solicited local and systemic reactions (eg, injection site pain, fatigue, headache). These solicited reactions do not include allergic reactions or anaphylaxis; however, v-safe does allow participants to enter free-text information about their postvaccination experience and asks about adverse health events (eg, received medical care). Medically attended events are followed up on through active telephone outreach; future analyses will address these adverse vaccine experiences. This report describes information on solicited local and systemic reactogenicity reported to v-safe on days 0 to 7 after each dose of vaccine from December 14, 2020, through February 28, 2021. Responses were limited to individuals who were vaccinated by February 21, 2021, to allow a 7-day reporting period after the day of vaccination. Preliminary data from v-safe through January 13, 2021, have been previously reported.[7] This activity was reviewed by the CDC and was conducted consistent with applicable federal law and CDC policy (see Additional Information).

Table. Solicited Local and Systemic Reactions[a] to mRNA-Based COVID-19 Vaccines Reported 0 to 7 Days After Vaccination—Centers for Disease Control and Prevention V-safe Surveillance System, December 14, 2020, to February 28, 2021

Reaction	No. (%)					
	Dose 1			Dose 2		
	Both vaccines (N = 3 643 918)	Pfizer-BioNTech (n = 1 659 724)	Moderna (n = 1 984 194)	Both vaccines (N = 1 920 872)	Pfizer-BioNTech (n = 971 375)	Moderna (n = 949 497)
Any injection site reaction	2 550 710 (70.0)	1 085 242 (65.4)	1 465 468 (73.9)	1 443 899 (75.2)	666 635 (68.6)	777 264 (81.9)
Pain	2 472 373 (67.8)	1 055 604 (63.6)	1 416 769 (71.4)	1 389 629 (72.3)	645 917 (66.5)	743 712 (78.3)
Redness	204 097 (5.6)	56 780 (3.4)	147 317 (7.4)	240 265 (12.5)	57 956 (6.0)	182 309 (19.2)
Swelling	379 539 (10.4)	110 077 (6.6)	269 462 (13.6)	348 986 (18.2)	100 430 (10.3)	248 556 (26.2)
Itching	197 441 (5.4)	62 486 (3.8)	134 955 (6.8)	214 658 (11.2)	60 946 (6.3)	153 712 (16.2)
Any systemic reaction[a]	1 823 068 (50.0)	797 410 (48.0)	1 025 658 (51.7)	1 333 931 (69.4)	623 746 (64.2)	710 185 (74.8)
Fatigue	1 127 638 (30.9)	483 146 (29.1)	644 492 (32.5)	1 034 462 (53.9)	464 659 (47.8)	569 803 (60.0)
Headache	943 607 (25.9)	409 359 (24.7)	534 248 (26.9)	897 005 (46.7)	392 266 (40.4)	504 739 (53.2)
Myalgia	705 100 (19.4)	281 743 (17.0)	423 357 (21.3)	845 314 (44.0)	357 381 (36.8)	487 933 (51.4)
Chills	321 009 (8.8)	116 034 (7.0)	204 975 (10.3)	600 354 (31.3)	220 831 (22.7)	379 523 (40.0)
Fever	314 676 (8.6)	116 951 (7.0)	197 725 (10.0)	566 112 (29.5)	208 976 (21.5)	357 136 (37.6)
Joint pain	317 034 (8.7)	123 319 (7.4)	193 715 (9.8)	492 031 (25.6)	192 926 (19.9)	299 105 (31.5)
Nausea	275 423 (7.6)	114 087 (6.9)	161 336 (8.1)	319 248 (16.6)	127 454 (13.1)	191 794 (20.2)
Vomiting	25 425 (0.7)	9966 (0.6)	15 459 (0.8)	31 056 (1.6)	11 276 (1.2)	19 780 (2.1)
Diarrhea	189 878 (5.2)	83 016 (5.0)	106 862 (5.4)	133 877 (7.0)	60 641 (6.2)	73 236 (7.7)
Abdominal pain	111 044 (3.0)	47 096 (2.8)	63 948 (3.2)	117 494 (6.1)	48 129 (5.0)	69 365 (7.3)
Rash outside of injection site	42 409 (1.2)	17 765 (1.1)	24 644 (1.2)	32 686 (1.7)	13 132 (1.4)	19 554 (2.1)

[a] Systemic reactions do not include allergic reactions or anaphylaxis.

Self-reported Local and Systemic Reactions Among V-safe Participants

By February 21, 2021, more than 46 million persons received at least 1 dose of an mRNA-based COVID-19 vaccine.[8] A total of 3 643 918 persons were enrolled in v-safe and completed at least 1 health survey within 7 days following their first vaccine dose; 1 920 872 v-safe participants reported receiving a second vaccine dose and completed at least 1 daily health survey within 7 days following the second dose. Solicited local and systemic reactions during days 0 to 7 after each dose were assessed.

Most v-safe participants reported an injection site reaction (dose 1: 70.0%; dose 2: 75.2%) or a systemic reaction (dose 1: 50.0%; dose 2: 69.4%) during days 0 to 7 after vaccination (**Table**). The most frequently reported solicited local and systemic reactions after the first dose of COVID-19 vaccine were injection site pain (67.8%), fatigue (30.9%), headache (25.9%), and myalgia (19.4%). Reactogenicity was substantially greater after the second dose for both vaccines, particularly for systemic reactions, including fatigue (53.9%), headache (46.7%), myalgia (44.0%), chills (31.3%), fever (29.5%), and joint pain (25.6%).

A greater percentage of participants who received the Moderna vaccine, compared with the Pfizer-BioNTech vaccine, reported reactogenicity; this pattern was more pronounced after the second dose (Table). When stratified by age (<65 vs ≥65 years), differences in reactogenicity by vaccine remained consistent with overall findings (data not shown). Local and systemic reactions were less commonly reported by v-safe participants 65 years and older compared with those younger than 65 years, but greater reactogenicity after the second dose was observed for both age groups (eFigure in the Supplement). For both doses of both vaccines, the percentage of v-safe participants who reported local and systemic reactions was highest on day 1 after vaccination and declined markedly through day 7.

The frequency of reported reactions was generally consistent with results observed in clinical trials.[4,5] Data from millions of v-safe participants indicate that injection site pain is common after both the first and second doses of either mRNA-based vaccine. Systemic reactions, including fatigue, headache, myalgia, chills, fever, and joint pain, occurred in participants after the first dose, although they were more frequently reported after the second dose among both Pfizer-BioNTech and Moderna vaccine recipients. Persons 65 years and older reported less reactogenicity than younger persons. Limitations of v-safe include voluntary participation via an opt-in smartphone-based system that includes less than 10% of vaccinated persons.

Although local and systemic reactions are expected and often transient, they may have the most immediate influence on patients' perceptions of the vaccination experience. Setting expectations with patients may alleviate some of the potential anxiety elicited by postvaccination reactogenicity. Clinicians should counsel vaccine recipients that these solicited local and systemic reactions are most commonly reported during the first day following their second dose; a short period before symptom resolution can be expected.[9]

ARTICLE INFORMATION

Author Affiliations: CDC COVID-19 Response Team, Atlanta, Georgia.

Corresponding Author: Johanna Chapin-Bardales, PhD, MPH, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30329 (wif3@cdc.gov).

Published Online: April 5, 2021. doi:10.1001/jama.2021.5374

Conflict of Interest Disclosures: Drs Chapin-Bardales, Gee, and Myers reported receiving nonfinancial technical support to build and maintain the v-safe infrastructure for data capture and messaging to participants from Oracle during the conduct of the study.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention (CDC). Mention of a product or company name is for identification purposes only and does not constitute endorsement by the CDC.

Additional Contributions: We thank investigators from the CDC COVID-19 Response Team and the CDC v-safe team, members of the Oracle v-safe development team, and v-safe participants who contributed to these data.

Additional Information: See eg, 45 CFR part 46.102(l)(2); 21 CFR part 56; 42 USC §241(d); 5 USC §552a; 44 USC §3501 et seq.

REFERENCES

1. Creech CB, Walker SC, Samuels RJ. SARS-CoV-2 vaccines. *JAMA*. 2021. doi:10.1001/jama.2021.3199

2. Oliver SE, Gargano JW, Marin M, et al. The Advisory Committee on Immunization Practices' interim recommendation for use of Pfizer-BioNTech COVID-19 vaccine: United States, December 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(50):1922-1924. doi:10.15585/mmwr.mm6950e2

3. Oliver SE, Gargano JW, Marin M, et al. The Advisory Committee on Immunization Practices' interim recommendation for use of Moderna COVID-19 vaccine: United States, December 2020. *MMWR Morb Mortal Wkly Rep*. 2021;69(5152):1653-1656. doi:10.15585/mmwr.mm695152e1

4. Polack FP, Thomas SJ, Kitchin N, et al; C4591001 Clinical Trial Group. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med*. 2020;383(27):2603-2615. doi:10.1056/NEJMoa2034577

5. Baden LR, El Sahly HM, Essink B, et al; COVE Study Group. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2021;384(5):403-416. doi:10.1056/NEJMoa2035389

6. V-safe protocol: version 2. Centers for Disease Control and Prevention. Published January 28, 2021. Accessed March 25, 2021. https://www.cdc.gov/vaccinesafety/pdf/V-safe-Protocol-508.pdf

7. Gee J, Marquez P, Su J, et al. First month of COVID-19 vaccine safety monitoring: United States, December 14, 2020-January 13, 2021. *MMWR Morb Mortal Wkly Rep*. 2021;70(8):283-288. doi:10.15585/mmwr.mm7008e3

8. Trends in number of COVID-19 vaccinations in the US. Centers for Disease Control and Prevention. Accessed March 20, 2021. https://covid.cdc.gov/covid-data-tracker/#vaccination-trends

9. Vaccines and immunizations: answering patients' questions. Centers for Disease Control and Prevention. Updated November 2, 2020. Accessed March 25, 2021. https://www.cdc.gov/vaccines/covid-19/hcp/answering-questions.html

Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial

Maheshi N Ramasamy, Angela M Minassian*, Katie J Ewer*, Amy L Flaxman*, Pedro M Folegatti*, Daniel R Owens*, Merryn Voysey*, Parvinder K Aley, Brian Angus, Gavin Babbage, Sandra Belij-Rammerstorfer, Lisa Berry, Sagida Bibi, Mustapha Bittaye, Katrina Cathie, Harry Chappell, Sue Charlton, Paola Cicconi, Elizabeth A Clutterbuck, Rachel Colin-Jones, Christina Dold, Katherine R W Emary, Sofiya Fedosyuk, Michelle Fuskova, Diane Gbesemete, Catherine Green, Bassam Hallis, Mimi M Hou, Daniel Jenkin, Carina C D Joe, Elizabeth J Kelly, Simon Kerridge, Alison M Lawrie, Alice Lelliott, May N Lwin, Rebecca Makinson, Natalie G Marchevsky, Yama Mujadidi, Alasdair P S Munro, Mihaela Pacurar, Emma Plested, Jade Rand, Thomas Rawlinson, Sarah Rhead, Hannah Robinson, Adam J Ritchie, Amy L Ross-Russell, Stephen Saich, Nisha Singh, Catherine C Smith, Matthew D Snape, Rinn Song, Richard Tarrant, Yrene Themistocleous, Kelly M Thomas, Tonya L Villafana, Sarah C Warren, Marion E E Watson, Alexander D Douglas*, Adrian V S Hill*, Teresa Lambe*, Sarah C Gilbert*, Saul N Faust*, Andrew J Pollard*, and the Oxford COVID Vaccine Trial Group*

Summary

Background Older adults (aged ≥70 years) are at increased risk of severe disease and death if they develop COVID-19 and are therefore a priority for immunisation should an efficacious vaccine be developed. Immunogenicity of vaccines is often worse in older adults as a result of immunosenescence. We have reported the immunogenicity of a novel chimpanzee adenovirus-vectored vaccine, ChAdOx1 nCoV-19 (AZD1222), in young adults, and now describe the safety and immunogenicity of this vaccine in a wider range of participants, including adults aged 70 years and older.

Methods In this report of the phase 2 component of a single-blind, randomised, controlled, phase 2/3 trial (COV002), healthy adults aged 18 years and older were enrolled at two UK clinical research facilities, in an age-escalation manner, into 18–55 years, 56–69 years, and 70 years and older immunogenicity subgroups. Participants were eligible if they did not have severe or uncontrolled medical comorbidities or a high frailty score (if aged ≥65 years). First, participants were recruited to a low-dose cohort, and within each age group, participants were randomly assigned to receive either intramuscular ChAdOx1 nCoV-19 ($2·2\times10^{10}$ virus particles) or a control vaccine, MenACWY, using block randomisation and stratified by age and dose group and study site, using the following ratios: in the 18–55 years group, 1:1 to either two doses of ChAdOx1 nCoV-19 or two doses of MenACWY; in the 56–69 years group, 3:1:3:1 to one dose of ChAdOx1 nCoV-19, one dose of MenACWY, two doses of ChAdOx1 nCoV-19, or two doses of MenACWY; and in the 70 years and older, 5:1:5:1 to one dose of ChAdOx1 nCoV-19, one dose of MenACWY, two doses of ChAdOx1 nCoV-19, or two doses of MenACWY. Prime-booster regimens were given 28 days apart. Participants were then recruited to the standard-dose cohort ($3·5–6·5\times10^{10}$ virus particles of ChAdOx1 nCoV-19) and the same randomisation procedures were followed, except the 18–55 years group was assigned in a 5:1 ratio to two doses of ChAdOx1 nCoV-19 or two doses of MenACWY. Participants and investigators, but not staff administering the vaccine, were masked to vaccine allocation. The specific objectives of this report were to assess the safety and humoral and cellular immunogenicity of a single-dose and two-dose schedule in adults older than 55 years. Humoral responses at baseline and after each vaccination until 1 year after the booster were assessed using an in-house standardised ELISA, a multiplex immunoassay, and a live severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) microneutralisation assay (MNA_{80}). Cellular responses were assessed using an ex-vivo IFN-γ enzyme-linked immunospot assay. The coprimary outcomes of the trial were efficacy, as measured by the number of cases of symptomatic, virologically confirmed COVID-19, and safety, as measured by the occurrence of serious adverse events. Analyses were by group allocation in participants who received the vaccine. Here, we report the preliminary findings on safety, reactogenicity, and cellular and humoral immune responses. This study is ongoing and is registered with ClinicalTrials.gov, NCT04400838, and ISRCTN, 15281137.

Findings Between May 30 and Aug 8, 2020, 560 participants were enrolled: 160 aged 18–55 years (100 assigned to ChAdOx1 nCoV-19, 60 assigned to MenACWY), 160 aged 56–69 years (120 assigned to ChAdOx1 nCoV-19: 40 assigned to MenACWY), and 240 aged 70 years and older (200 assigned to ChAdOx1 nCoV-19: 40 assigned to MenACWY). Seven participants did not receive the boost dose of their assigned two-dose regimen, one participant received the incorrect vaccine, and three were excluded from immunogenicity analyses due to incorrectly labelled samples. 280 (50%) of 552 analysable participants were female. Local and systemic reactions were more common in participants given ChAdOx1 nCoV-19 than in those given the control vaccine, and similar in nature to those previously reported

Lancet 2020; 396: 1979–93

Published Online November 19, 2020 https://doi.org/10.1016/S0140-6736(20)32466-1

This online publication has been corrected. The corrected version first appeared at thelancet.com on December 17, 2020, and further corrections have been made on April 8, 2021

See Comment page 1942

*Contributed equally

Oxford Vaccine Group, Department of Paediatrics (M N Ramasamy DPhil, M Voysey DPhil, P K Aley PhD, S Bibi PhD, E A Clutterbuck PhD, R Colin-Jones MSc, C Dold PhD, K R W Emary BM BCh, S Kerridge MSc, A Lelliott BMBS, N G Marchevsky MSc, Y Mujadidi MSc, E Plested, S Rhead MBChB, N Singh DPhil, C C Smith PhD, M D Snape MD, R Song MD, Prof A J Pollard FMedSci), **The Jenner Institute** (A M Minassian DPhil, K J Ewer PhD, A L Flaxman DPhil, P M Folegatti MD, B Angus MD, G Babbage MPhil, S Belij-Rammerstorfer PhD, M Bittaye PhD, P Cicconi PhD, S Fedosyuk PhD, M Fuskova MSc, M M Hou MRCPCH, D Jenkin MRCP, C C D Joe PhD, A M Lawrie PhD, R Makinson MBiol, T Rawlinson DPhil, A J Ritchie PhD, Y Themistocleous MBChB, M E E Watson PhD, A D Douglas DPhil, Prof A V S Hill FMedSci, T Lambe PhD, Prof S C Gilbert PhD), **and Clinical**

(injection-site pain, feeling feverish, muscle ache, headache), but were less common in older adults (aged ≥56 years) than younger adults. In those receiving two standard doses of ChAdOx1 nCoV-19, after the prime vaccination local reactions were reported in 43 (88%) of 49 participants in the 18–55 years group, 22 (73%) of 30 in the 56–69 years group, and 30 (61%) of 49 in the 70 years and older group, and systemic reactions in 42 (86%) participants in the 18–55 years group, 23 (77%) in the 56–69 years group, and 32 (65%) in the 70 years and older group. As of Oct 26, 2020, 13 serious adverse events occurred during the study period, none of which were considered to be related to either study vaccine. In participants who received two doses of vaccine, median anti-spike SARS-CoV-2 IgG responses 28 days after the boost dose were similar across the three age cohorts (standard-dose groups: 18–55 years, 20 713 arbitrary units [AU]/mL [IQR 13 898–33 550], n=39; 56–69 years, 16 170 AU/mL [10 233–40 353], n=26; and ≥70 years 17 561 AU/mL [9705–37 796], n=47; p=0·68). Neutralising antibody titres after a boost dose were similar across all age groups (median MNA_{80} at day 42 in the standard-dose groups: 18–55 years, 193 [IQR 113–238], n=39; 56–69 years, 144 [119–347], n=20; and ≥70 years, 161 [73–323], n=47; p=0·40). By 14 days after the boost dose, 208 (>99%) of 209 boosted participants had neutralising antibody responses. T-cell responses peaked at day 14 after a single standard dose of ChAdOx1 nCoV-19 (18–55 years: median 1187 spot-forming cells [SFCs] per million peripheral blood mononuclear cells [IQR 841–2428], n=24; 56–69 years: 797 SFCs [383–1817], n=29; and ≥70 years: 977 SFCs [458–1914], n=48).

Interpretation ChAdOx1 nCoV-19 appears to be better tolerated in older adults than in younger adults and has similar immunogenicity across all age groups after a boost dose. Further assessment of the efficacy of this vaccine is warranted in all age groups and individuals with comorbidities.

Funding UK Research and Innovation, National Institutes for Health Research (NIHR), Coalition for Epidemic Preparedness Innovations, NIHR Oxford Biomedical Research Centre, Thames Valley and South Midlands NIHR Clinical Research Network, and AstraZeneca.

Biomanufacturing Facility (C Green PhD, R Tarrant PhD), **Nuffield Department of Medicine, and Oxford Centre for Clinical Tropical Medicine and Global Health** (H Robinson DipHE), **University of Oxford, Oxford, UK; NIHR Clinical Research Facility, University Hospital Southampton NHS Trust, Southampton, UK** (D R Owens MRCPCH, L Berry BSc, H Chappell MBBS, D Gbesemete MRCPCH, M N Lwin MSc, A P S Munro MRCPCH, M Pacurar MD, J Rand BA, A L Ross-Russell MA, S Saich BA, S C Warren MBBC); **Paediatric Medicine, University of Southampton, Southampton, UK** (K Cathie MD); **National Infection Service, Public Health England, Porton Down, Salisbury, UK** (S Charlton PhD, B Hallis PhD, K M Thomas PhD); **NIHR Oxford Biomedical Research Centre, Oxford, UK** (E A Clutterbuck, C Dold, S Rhead, H Robinson, A D Douglas, Prof A V S Hill, T Lambe, Prof S C Gilbert, Prof A J Pollard); **AstraZeneca BioPharmaceuticals Research and Development, Washington, DC, USA** (E J Kelly PhD); **AstraZeneca BioPharmaceuticals Research and Development , Bethesda, MA, USA** (T L Villafana PhD); **Division of Infectious Diseases, Boston Children's Hospital, Boston, MA, USA** (R Song); **and NIHR Southampton Clinical Research Facility and Biomedical Research Centre, University Hospital Southampton NHS Trust and Faculty of Medicine and Institute for Life Sciences, University of Southampton, Southampton, UK** (Prof S N Faust FRCPCH)

Correspondence to:
Dr Maheshi N Ramasamy, Department of Paediatrics, University of Oxford, Oxford OX3 9DU, UK
maheshi.ramasamy@paediatrics.ox.ac.uk

Introduction

As of Nov 13, 2020, over 52 million people have been diagnosed with COVID-19 worldwide, with over 1·2 million confirmed deaths.[1] Severe COVID-19 is more common in adults aged 70 years and older and in individuals with comorbidities such as hypertension, diabetes, cardiovascular disease, and chronic respiratory disease.[2] A safe and effective vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) will be an important tool in controlling the global COVID-19 pandemic. Although there are no licensed vaccines against COVID-19, 48 potential vaccine candidates based on a variety of platforms including lipid nanoparticle mRNA, DNA, adjuvanted protein, inactivated virus particles, and non-replicating viral vectors are in clinical trials (of which 11 candidates are in phase 3 trials) and a further 164 candidates are in preclinical testing.[3]

The WHO global target product profile of critical characteristics for prequalification of a COVID-19 vaccine requires candidates to be targeted at the most at-risk groups, including older adults; have a favourable safety profile; provide efficacy as measured by prevention of virologically confirmed disease or transmission, or both; and to provide at least 6 months of protection for individuals at ongoing risk of exposure to SARS-CoV-2.[4] On Sept 25, 2020, the UK Joint Committee on Vaccination and Immunisation (JCVI) gave interim recommendations for the national prioritisation of COVID-19 vaccines.[5] The following groups were provisionally prioritised: first, older adults living in residential care homes and residential care home workers; second, all adults aged 80 years or older and health-care and social-care workers; and third, all adults aged 75 years and older. However, the JCVI acknowledged that this priority ranking could change substantially if the first available vaccines were not considered safe or effective in older adults. Similar recommendations have also been made by the US Advisory Committee on Immunization Practices.[6]

Immunosenescence refers to the gradual deterioration and decline of the immune system brought on by ageing. Age-dependent differences in the functionality and availability of T-cell and B-cell populations are thought to have a key role in the decrease of immune response.[7] There has been a drive to develop vaccines and adjuvant formulations tailored for older adults to overcome this diminished immune response after vaccination. Assessment of immune responses in older adults is therefore essential in the development of COVID-19 vaccines that could protect this susceptible population.

The spike protein of SARS-CoV-2 binds to ACE2 receptors on target cells during viral entry. Analysis of convalescent patients suggests that the spike protein is an immunodominant antigen, eliciting both antibody and T-cell responses.[8] Most COVID-19 candidate vaccines have been developed to induce anti-spike protein immune responses. Clinical trials using several different vaccine platforms including mRNA,[9,10] adenoviral vectored vaccines,[11,12] inactivated virus,[13,14] and adjuvanted

Research in context

Evidence before this study

We searched PubMed for research articles published from database inception until Nov 13, 2020, with no language restrictions, using the terms "SARS-CoV-2", "vaccine", AND "clinical trial". We identified published clinical trial data on eight other vaccine candidates. Two recombinant viral vectored vaccines have been tested in clinical trials. A single dose adenovirus (Ad) 5 vector-based vaccine (CanSino Biological/Beijing Institute of Biotechnology, China) elicited neutralising antibodies and T-cell responses in a dose-dependent manner, but was less immunogenic in individuals older than 55 years. A heterologous prime-boost Ad5/Ad26-vectored vaccine schedule (Gamaleya Research Institute, Russia) generated neutralising antibody and cellular responses in adults younger than 60 years. Two nucleoside-modified mRNA vaccine candidates using a two-dose regimen were tested in adults aged 18–55 years and 65–85 years, and generated neutralising antibodies in both age groups in a dose-dependent manner, although immunogenicity decreased with age (Pfizer/BioNTech, USA). Another mRNA vaccine (Moderna, USA) was given to adults older than 56 years. The vaccine was tolerated, with neutralising antibodies induced in a dose-dependent manner, which increased after a second dose. Neutralising antibody responses with this mRNA vaccine appeared to be similar in adults older than 56 years to those aged 18–55 years who also received the vaccine. Two inactivated viral vaccines have also shown neutralising antibody responses in a dose-dependent manner in adults aged 18–59 years (Wuhan Institute Biological Products/SinoPharm, China) or adults aged 18–59 and 60 years and older (Beijing Institute Biological products/SinoPharm, China), with the second showing lower neutralising antibody titres in older adults after two doses. Finally, a clinical trial of a nanoparticle vaccine composed of adjuvanted trimeric severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike glycoproteins (Novavax, USA) reported results of a two-dose schedule given 3 weeks apart in healthy adults younger than 60 years. This vaccine was well tolerated and induced neutralisation responses that exceeded those measured in serum samples from convalescent symptomatic patients.

Added value of this study

This study is the fifth published clinical trial of a vaccine against SARS-CoV-2 tested in an older adult population (aged 18–55 years, 56–69 years, and ≥70 years). The vaccine was safe and well tolerated, with reduced reactogenicity in older adults. Antibody responses against the SARS-CoV-2 spike protein were induced in all age groups and were boosted and maintained at 28 days after booster vaccination, including in the 70 years and older group. Cellular immune responses were also induced in all age and dose groups, peaking at day 14 after vaccination.

Implications of all the available evidence

The populations at greatest risk of serious COVID-19 include people with coexisting health conditions and older adults. The immune correlates of protection against SARS-CoV-2 have not yet been determined, but neutralising antibodies are thought to be associated with protection, and in a COVID-19 non-human primate challenge model, neutralising antibody responses correlated with protection. These findings have led to the use of neutralisation assays to assess immune responses in recent human COVID-19 vaccine trials. Immunisation with ChAdOx1 nCoV-19 results in development of neutralising antibodies against SARS-CoV-2 in almost 100% of participants including older adults without severe comorbidities, with higher levels in boosted compared with non-boosted groups. Further assessment of the efficacy of this vaccine is warranted in all age groups and individuals with comorbidities.

spike glycoprotein[15] have shown neutralising antibody responses after immunisation.

Replication-deficient adenovirus vectors containing a pathogen-specific transgene have been used as novel vaccines because of their ability to induce strong humoral and cellular responses.[16] However, pre-existing immunity might reduce the immunogenicity of vectors derived from human viruses; hence, use of simian adenoviruses might be preferable. ChAdOx1 nCoV-19 (AZD1222) is a replication-defective chimpanzee adenovirus-vectored vaccine expressing the full-length SARS-CoV-2 spike glycoprotein gene (GenBank accession number MN908947). Vaccination of rhesus macaques with a single dose of ChAdOx1 nCoV-19 generates humoral and cellular immune responses and protects from lower respiratory infection after subsequent challenge with SARS-CoV-2.[17] Preliminary results of a phase 1/2 clinical trial of ChAdOx1 nCoV-19 in adults aged 18–55 years show that the vaccine is well tolerated and generates robust neutralising antibody and cellular immune responses against the spike glycoprotein.[18] Here we present the safety and immunogenicity results of a phase 2 component of a phase 2/3 multicentre study using ChAdOx1 nCoV-19 at two different doses, in adults including those aged 56–69 years and 70 years and older, and in a one-dose or two-dose regimen.

Methods

Study design and participants

In this continuing single-blind, multicentre, randomised, controlled, phase 2/3 trial, the safety and efficacy of the ChAdOx1 nCoV-19 vaccine is being assessed, with sequential age-escalation immunogenicity substudies being done in older age groups. The study is being run at 20 centres in the UK (listed in the appendix [pp 84–87]). Here we report selected results from the phase 2 component of the trial and for which participants were enrolled at two sites in the UK: the Oxford Vaccine Centre, Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford (Oxford) and the NIHR

See Online for appendix

Southampton Clinical Research Facility, University Hospital Southampton NHS Foundation Trust (Southampton). Data on the participants from the phase 3 component will be published elsewhere.

We recruited participants in an age-escalation manner. We recruited adults aged 18–55 years, then adults aged 56–69 years, and then adults aged 70 years and older, without severe or uncontrolled medical comorbidities, as defined in the clinical study plan (appendix pp 48–54), through local advertisements. Participants aged 65 years and older with a Dalhousie Clinical Frailty Score of 4 or higher were excluded.[19]

Participants were enrolled into one of ten different groups. Recruitment was sequential with low-dose groups recruited first and standard-dose cohorts recruited after a protocol amendment was approved on June 5, 2020, that incorporated the new higher dose level. For the first stage of recruitment, participants aged 18–55 years were recruited to the low-dose group. Subsequently we recruited participants aged 56–69 years, and further extension to recruit those aged 70 years and older only occurred after safety review by the independent Data Safety Monitoring Board (DSMB). A minimum of 2 weeks of safety and immunogenicity data were reviewed by the DSMB before recruitment to each successive age cohort. The 18–55 years groups received two doses of vaccine and were randomly assigned to receive either the experimental vaccine or the control vaccine. The 56–69 years and 70 years and older groups were randomly assigned to receive either one dose or two doses of vaccine and were then randomly assigned to receive the experimental vaccine or the control vaccine. The same process was repeated with recruitment and randomisation for the standard-dose cohorts after review by the DSMB. All participants underwent a screening visit in which a full medical history, targeted examination, blood test for SARS-CoV-2 exposure, and a urinary pregnancy test in women of childbearing potential were done. Volunteers who were seropositive to SARS-CoV-2 before enrolment were excluded from participating in all groups, apart from those in the 18–55 years standard-dose cohort. Additionally, all participants included in this phase 2 component of the study, apart from those in the 18–55 years low-dose group, had additional safety tests (blood tests for HIV, hepatitis B and C serology, full blood count, and kidney and liver function tests). Full details of eligibility criteria are in the trial protocol (appendix pp 135–38).

Written informed consent was obtained from all participants, and the trial is being done in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The study was sponsored by the University of Oxford (Oxford, UK) and approved in the UK by the Medicines and Healthcare products Regulatory Agency (reference 21584/0428/001-0001) and the South-Central Berkshire Research Ethics Committee (reference 20/SC/0179). Vaccine use was authorised by Genetically Modified Organisms Safety Committees at each participating site. An independent DSMB reviewed all interim safety reports. A copy of the protocol is included in the appendix (pp 83–212).

Randomisation and masking

Participants were randomly assigned to receive either the ChAdOx1 nCoV-19 vaccine or the quadrivalent MenACWY protein-polysaccharide conjugate vaccine. MenACWY was used as a comparator vaccine rather than a saline placebo to maintain masking of participants who had local or systemic reactions. Participants aged 18–55 years were randomly assigned (1:1) in the low-dose cohort and (5:1) in the standard-dose cohort to receive either ChAdOx1 nCoV-19 or MenACWY. For both 18–55 years cohorts, participants were given two doses of study vaccine. Participants aged 56–69 years were randomly assigned (3:1:3:1) to one dose of ChAdOx1 nCoV-19, one dose of MenACWY, two doses of ChAdOx1 nCoV-19, or two doses of MenACWY. Participants aged 70 years or older were randomly assigned (5:1:5:1) to one dose of ChAdOx1 nCoV-19, one dose of MenACWY, two doses of ChAdOx1 nCoV-19, or two doses of MenACWY.

Randomisation lists, using block randomisation stratified by age and dose group and study site, were generated by the study statistician (MV). Block sizes were chosen to align with the age group and dose group sizes. Computer randomisation was done with full allocation concealment within the secure web platform used for the study electronic case report form (REDCap version 9.5.22). The trial staff administering the vaccine prepared vaccines out of sight of the participants and syringes were covered with an opaque material until ready for administration to ensure masking of participants. Participants, clinical investigators, and the laboratory team remained masked to group allocation for the duration of the study. However, trial staff administering the vaccine were unmasked.

Procedures

In the previous phase 1/2 study,[18] a single standard dose of 5×10^{10} virus particles of ChAdOx1 nCoV-19 was used, based on previous experience with a ChAdOx1 Middle East respiratory syndrome (MERS) construct. In this study, we assessed a lower dose of $2\cdot2\times10^{10}$ virus particles and a standard dose of $3\cdot5–6\cdot5\times10^{10}$ virus particles in adults of different age cohorts. Due to the need to rapidly produce large numbers of doses of vaccine manufactured using Good Manufacturing Practice to allow timely enrolment into the phase 2/3 clinical trial, two different batches of vaccine were used in this study: one manufactured and vialed by Advent (Pomezia, Italy), and one manufactured by COBRA Biologics (Keele, UK) and vialed by Symbiosis (Stirling, UK). Both were manufactured according to Good Manufacturing Practice and approved by the regulatory agency in the UK, the Medicines and Healthcare

products Regulatory Agency. The 18–55 years standard-dose cohort received vaccine manufactured by COBRA Biologics for both first (ie, prime) and second (ie, boost) doses and all other cohorts received prime and boost doses, as randomised, manufactured by Advent. Analytical assessment of the batches indicates that the batches are comparable. Formal batch-to-batch comparison studies are ongoing and results will be reported when available.

ChAdOx1 nCoV-19 was administered as a single-dose or two-dose regimen (28 days apart) at either the low dose ($2·2\times10^{10}$ virus particles) or the standard dose ($3·5–6·5\times10^{10}$ virus particles). It was administered as a single intramuscular injection into the deltoid, according to specific study standard operating procedures. The MenACWY vaccine was provided by the UK Department of Health and Social Care and administered as per summary of product characteristics at the standard dose.[20] Depending on the batch used for vaccination, the injection volume for the low dose of ChAdOx1 nCoV-19 was either 0·22 mL or 0·5 mL. The injection volume used for the standard dose of ChAdOx1 nCoV-19 and MenACWY was 0·5 mL.

Safety data from animal studies and our previous phase 1/2 clinical trial[18] of ChAdOx1 nCoV-19 were reviewed before recruitment of participants. Volunteers were considered enrolled into the trial at the point of vaccination. Participants were observed in the clinic for a minimum of 15 min after the vaccination procedure in case of any immediate adverse events.

Participants from each group were instructed to complete a diary card to record solicited local and systemic adverse reactions for 7 days after each dose. Protocol-defined solicited local adverse events included injection-site pain, tenderness, warmth, redness, swelling, induration, and itch, and solicited systemic adverse events included malaise, muscle ache, joint pain, fatigue, nausea, headache, chills, feverishness (ie, a self-reported feeling of having a fever), and objective fever (defined as an oral temperature of 38°C or higher). All participants were given an emergency 24-h telephone number to contact the on-call study physician as required. Serious adverse events will be recorded throughout the follow-up period of 1 year after the last dose of vaccine.

Severity of adverse events was graded with the following criteria: mild (transient or mild discomfort for <48 h, no interference with activity, and no medical intervention or therapy required), moderate (mild-to-moderate limitation in activity, and no or minimal medical intervention or therapy required), severe (substantial limitation in activity and medical intervention or therapy required), or potentially life-threatening (requires assessment in emergency department or admission to hospital). All participants in the 56–69 years and 70 years and older groups and participants in the 18–55 years standard-dose group had clinical and immunogenicity assessments at 0, 7, 14, and 28 days after their prime and booster vaccinations. Participants in the 18–55 years low-dose group had clinical and immunogenicity assessments at baseline, immediately before the boost dose, and at 14 and 28 days after their booster vaccination.

Humoral responses at baseline and after vaccination were assessed using Meso Scale Discovery multiplexed immunoassay against spike and receptor binding domain [RBD], a standardised total IgG ELISA against trimeric SARS-CoV-2 spike protein, and a live SARS-CoV-2 microneutralisation assay MNA_{80}, which was done at Public Health England (Porton Down, UK), as described previously.[18] Cellular responses were assessed using an ex-vivo IFN-γ enzyme-linked immunospot (ELISpot) assay to enumerate antigen-specific T cells.[18] Neutralising antibodies to the ChAdOx1 vector were measured using a secreted embryonic alkaline phosphatase (SEAP)-reporter assay, which measures the reciprocal of the serum dilution required to reduce in-vitro expression of vector-expressed SEAP by 50%, 24 h after transduction.[21] Due to the labour-intensive nature of neutralisation assays, we prioritised analysis of samples from the ChAdOx1 nCoV-19 groups, randomly selecting more samples from ChAdOx1 nCoV-19 participants than control samples to be sent for blinded analysis.

Outcomes

The coprimary outcomes of the trial are to assess efficacy as measured by the number of cases of symptomatic, virologically confirmed COVID-19 and safety of the vaccine as measured by the occurrence of serious adverse events. Secondary outcomes include safety, reactogenicity, and immunogenicity profiles of ChAdOx1 nCoV-19 in older adults (aged 56–69 years and ≥70 years), efficacy against severe and non-severe COVID-19, death, and seroconversion against non-spike proteins. A full list of secondary and tertiary outcomes is in the protocol (pp 118–24).

Here we report preliminary results for selected secondary endpoints, comparing local and systemic reactogenicity and cellular and humoral immunogenicity of ChAdOx1 nCoV-19 between different age groups, after one or two doses and at low or standard dose. Efficacy analyses are not included in this report.

Statistical analysis

We present safety endpoints as frequencies (%) with 95% binomial exact CIs. We present immunological endpoints as medians and IQR. Analyses were by group allocation in participants who received the vaccine.

We did comparisons across the three age groups (aged 18–55 years, aged 56–69 years, and aged ≥70 years) using Kruskal-Wallis tests within each dose level of the vaccine (low dose or standard dose) for antibody responses or unadjusted analysis of variance applied to log-transformed values for neutralisation titres. We did comparisons between low-dose and standard-dose groups using Wilcoxon rank sum tests (antibody

response) or independent samples Student's *t* test applied to log-transformed values for neutralisation titres. We present unadjusted p values for a small number of statistical comparisons to avoid issues of multiplicity. To assess the association between responses on different assays, we used unadjusted linear regression to analyse log-transformed values after baseline.

Sample sizes were nominal for these immunogenicity subgroups and no power calculations were done.

We did all statistical analyses using SAS version 9.4 and R version 3.6.1 or later. This study is registered with ClinicalTrials.gov, NCT04400838, and with ISRCTN, 15281137.

Role of the funding source

AstraZeneca reviewed the data from the study and the final manuscript before submission, but the authors retained editorial control. All other funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between May 30 and Aug 8, 2020, 560 participants were enrolled in the study and randomly assigned to the experimental vaccine or control vaccine group: 160 participants aged 18–55 years (100 assigned to ChAdOx1 nCoV-19, 60 assigned to MenACWY), 160 aged 56–69 years (120 assigned to ChAdOx1 nCoV-19, 40 assigned to MenACWY), and 240 aged 70 years and older (200 assigned to ChAdOx1 nCoV-19, 40 assigned to MenACWY). Full details on randomisation are in figure 1. All participants randomly assigned to treatment were vaccinated. One participant (in the 18–55 years low-dose group) received the incorrect vaccine after randomisation and was excluded from analysis. Seven participants randomly assigned to receive two doses of vaccine chose not to continue with the boost dose and were excluded from further analyses. Three participants were excluded from immunology analyses due to incorrectly labelled samples (either incorrect participant identification numbers or incorrect timepoints noted on the label, or both; figure 1). The baseline characteristics of the participants eligible for inclusion in the analysis in each group are shown in the table. Participants 70 years and older were recruited from the NIHR Southampton Clinical Research Facility, University Hospital Southampton NHS Foundation Trust. All other participants were recruited at the Oxford Vaccine Centre, Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford. Among the analysed population, 280 (50%) of 552 participants were female. 524 (95%) of 552 participants identified as white, and 540 (98%) were non-smokers. A large proportion of health-care workers who were predominantly female were enrolled in the 18–55 years and 56–69 years age groups. The median age in the 18–55 years group was 43·0 years (IQR 33·6–48·0), in the 56–69 years group was 60·0 years (57·5–63·0) and in the 70 years and older group was 73·0 years (71·0–76·0). The median age in the 70 years and older groups ranged from 73 years to 74 years across dosing groups, with the oldest participants aged 83 years.

The following results for local and systemic adverse reactions are all for participants who were randomly assigned to receive two doses of vaccine. Injection-site pain and tenderness were the most common solicited local adverse reactions and occurred most frequently in the first 48 h after vaccination (data for standard-dose regimen shown in figure 2; data for the low-dose groups and control groups are shown in the appendix [pp 7, 9, 19–21]). In those aged 56 years or older, a standard dose of ChAdOx1 nCoV-19, whether the prime or boost vaccination, elicited a greater number of local or systemic reactions than did MenACWY. The difference was less clear with the low-dose vaccine in the 56–69 years and 70 years and older groups, and the number of participants in the control groups was small (appendix p 30). At least one local symptom was reported after the prime vaccination with standard-dose ChAdOx1 nCoV-19 by 43 (88%) of 49 participants in the 18–55 years group, 22 (73%) of 30 in the 56–69 years group, and 30 (61%) of 49 in the 70 years and older group (appendix p 29). Similar proportions of local symptoms were reported after the boost vaccination with the standard dose of ChAdOx1 nCoV-19, with 37 (76%) of 49 participants in the 18–55 years group, 21 (72%) of 29 in the 56–69 years group, and 27 (55%) of 49 in the 70 years and older group reporting at least one local symptom. A similar pattern was seen across the age groups in participants after their prime vaccination with low-dose ChAdOx1 nCoV-19 and after the boost vaccination with the low-dose vaccine, but with fewer total adverse reactions than in the standard-dose groups (appendix pp 7, 9, 19–21). No severe local symptoms were reported by recipients of ChAdOx1 nCoV-19. In the two-dose control groups, across both the low-dose and standard-dose cohorts, local symptoms were reported by 33 (57%) of 58 participants in the 18–55 years group, five (25%) of 20 in the 56–69 years group, and seven (35%) of 20 in the 70 years and older group after the prime vaccination with MenACWY, and by 50 (86%) of 58 in the 18–55 years group, seven (37%) of 19 in the 56–69 years group, and four (20%) of 20 in the 70 years and older group after the boost vaccination with MenACWY (appendix p 29). Data for participants randomly assigned to receive only one dose of vaccine were similar to the data after a prime dose of vaccine in the two-dose groups (data not shown).

Fatigue, headache, feverishness, and myalgia were the most commonly solicited systemic adverse reactions (data for the standard-dose groups are shown in figure 3; data for the low-dose groups and control groups are shown in the appendix [pp 8, 10, 19–21]). At least one systemic symptom was reported after the prime

vaccination with the standard dose of ChAdOx1 nCoV-19 by 42 (86%) of 49 participants in the 18–55 years group, 23 (77%) of 30 in the 56–69 years group, and 32 (65%) of 49 in the 70 years and older group (appendix p 29). The severity of symptoms reported in the standard-dose groups was reduced after the boost vaccination, with only one (1%) of 127 participants reporting a severe reaction compared with seven (5%) of 128 participants after the prime vaccination. At least one systemic adverse reaction after the boost vaccination of standard dose of ChAdOx1

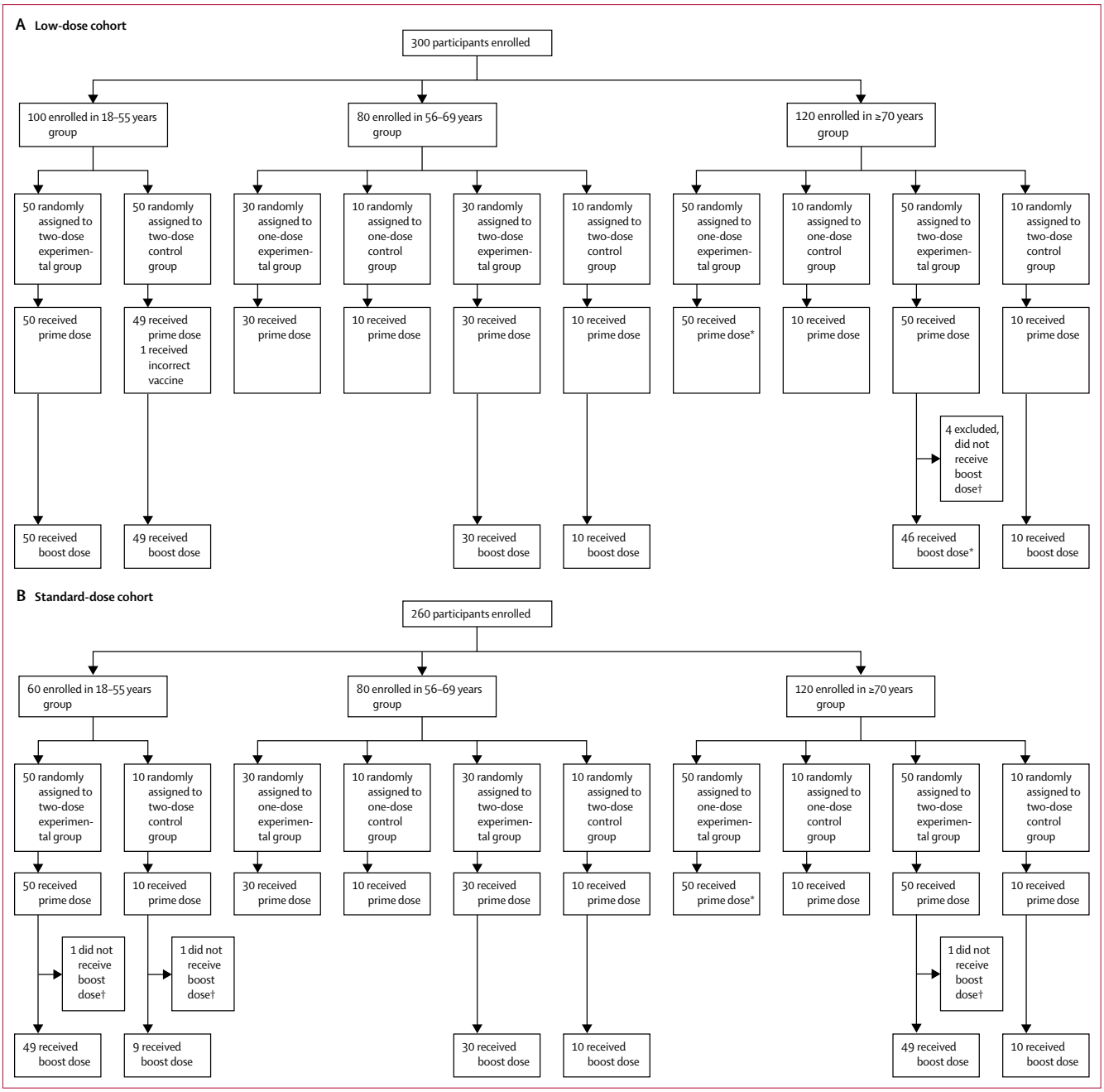


Figure 1: **Study profile for the low-dose (A) and standard-dose (B) cohorts**
*One participant excluded from immunogenicity analyses, due to mislabelling of laboratory sample. †Reasons for not receiving boost dose included that the participant moved away or was unavailable for visits, delay in receiving boost dose, or withdrawal of consent.

	Age 18–55 years		Age 56–69 years				Age ≥70 years			
	ChAdOx1 nCoV-19, two doses	MenACWY, two doses	ChAdOx1 nCoV-19, one dose	MenACWY, one dose	ChAdOx1 nCoV-19, two doses	MenACWY, two doses	ChAdOx1 nCoV-19, one dose	MenACWY, one dose	ChAdOx1 nCoV-19, two doses	MenACWY, two doses
Low dose										
Number enrolled	50	49	30	10	30	10	50	10	46	10
Sex										
Female	35 (70%)	28 (57%)	19 (63%)	4 (40%)	10 (33%)	8 (80%)	24 (48%)	6 (60%)	16 (35%)	6 (60%)
Male	15 (30%)	21 (43%)	11 (37%)	6 (60%)	20 (67%)	2 (20%)	26 (52%)	4 (40%)	30 (65%)	4 (40%)
Age, years, median (IQR, range)	44·5 (39·0–51·0, 22·0–54·0)	42·0 (32·0–48·0, 23·0–55·0)	60·0 (58·9–62·3, 56·0–69·0)	57·8 (56·3–60·8, 56·0–68·0)	60·4 (57·8–66·0, 56·0–69·4)	60·5 (58·3–63·9, 56·7–69·0)	73·5 (71·0–76·0, 69·0–83·0)	73·0 (70·0–74·0, 70·0–81·0)	73·0 (71·0–75·0, 70·0–82·0)	73·0 (71·2–74·0, 70·0–76·0)
BMI, kg/m², median (IQR, range)	24·6 (22·9–28·9, 19·4–45·1)	24·8 (21·6–27·7, 18·0–37·2)	25·0 (23·2–27·3, 20·2–37·6)	25·5 (22·5–27·3, 20·9–34·4)	25·9 (24·0–28·8, 21·3–36·6)	24·0 (23·2–26·0, 22·2–33·2)	26·0 (23·8–28·0, 20·0–36·0)	24·9 (22·3–26·9, 19·3–32·5)	26·0 (23·4–27·7, 19·4–42·1)	26·8 (24·3–29·5, 19·2–35·3)
Smoker	3 (6%)	1 (2%)	0	1 (10%)	2 (7%)	0	1 (2%)	0	1 (2%)	0
Alcohol drinker	44 (88%)	42 (86%)	28 (93%)	9 (90%)	26 (87%)	8 (80%)	43 (86%)	10 (100%)	43 (94%)	9 (90%)
Health-care worker	35 (70%)	26 (53%)	17 (57%)	7 (70%)	12 (40%)	4 (40%)	0	0	0	1 (10%)
Race or ethnicity										
White	48 (96%)	45 (92%)	30 (100%)	9 (90%)	27 (90%)	10 (100%)	50 (100%)	10 (100%)	45 (98%)	10 (100%)
Black or Black British	0	0	0	0	0	0	0	0	0	0
Asian or Asian British	2 (4%)	1 (2%)	0	0	2 (7%)	0	0	0	0	0
Mixed race or ethnicity	0	3 (6%)	0	0	0	0	0	0	1 (2%)	0
Other race or ethnicity*	0	0	0	1 (10%)	1 (3%)	0	0	0	0	0
Comorbidities										
Cardiovascular disease	4 (8%)	10 (20%)	5 (17%)	0	11 (37%)	0	14 (28%)	3 (30%)	16 (35%)	2 (20%)
Respiratory disease	12 (24%)	9 (18%)	7 (23%)	0	7 (23%)	0	6 (12%)	2 (20%)	6 (13%)	1 (10%)
Diabetes	0	0	0	0	0	1 (10%)	1 (2%)	0	2 (4%)	0
Standard dose										
Number enrolled	49	9	30	10	30	10	50	10	49	10
Sex										
Female	23 (47%)	7 (78%)	16 (53%)	3 (30%)	16 (53%)	5 (50%)	25 (50%)	1 (10%)	21 (43%)	2 (20%)
Male	26 (53%)	2 (22%)	14 (47%)	7 (70%)	14 (47%)	5 (50%)	25 (50%)	9 (90%)	28 (57%)	8 (80%)
Age, years, median (IQR, range)	39·0 (30·0–45·0, 19·0–55·0)	43·0 (35·8–50·0, 32·0–54·0)	59·0 (58·0–61·0, 56·0–69·0)	61·5 (57·5–63·8, 57·0–66·0)	59·5 (57·0–61·0, 56·0–67·0)	60·5 (57·9–61·0, 56·0–64·0)	74·0 (72·0–76·0, 70·0–80·0)	74·0 (71·0–75·5, 70·0–78·0)	73·0 (71·0–75·0, 70·0–83·0)	73·5 (72·2–74·8, 71·0–81·0)
BMI, kg/m², median (IQR, range)	26·9 (24·6–30·9, 20·2–39·7)	24·1 (23·8–25·6, 18·6–39·0)	26·7 (25·2–30·0, 18·6–36·8)	28·9 (25·6–30·2, 21·7–31·9)	24·0 (22·4–27·1, 19·9–33·5)	26·1 (23·6–27·7, 20·5–30·2)	25·1 (23·7–28·5, 17·5–32·6)	26·8 (25·8–28·5, 23·0–31·7)	27·1 (24·2–29·2, 20·3–40·2)	25·6 (24·1–29·3, 18·9–32·5)
Smoker	1 (2%)	0	0	0	0	1 (10%)	1 (2%)	0	0	0
Alcohol drinker	45 (92%)	6 (67%)	29 (97%)	10 (100%)	29 (97%)	10 (100%)	39 (78%)	9 (90%)	42 (86%)	9 (90·0%)
Health-care worker	13 (27%)	5 (56%)	10 (33%)	2 (20%)	12 (40%)	5 (50%)	2 (4%)	0	0	0
Race or ethnicity										
White	40 (82%)	7 (78%)	29 (97%)	10 (100%)	26 (87%)	9 (90%)	50 (100%)	10 (100%)	49 (100%)	10 (100%)
Black or Black British	1 (2%)	0	0	0	0	0	0	0	0	0
Asian or Asian British	7 (14%)	2 (22%)	0	0	4 (13%)	1 (10%)	0	0	0	0
Mixed race or ethnicity	0	0	0	0	0	0	0	0	0	0
Other race or ethnicity*	1 (2%)	0	1 (3%)	0	0	0	0	0	0	0
Comorbidities										
Cardiovascular disease	6 (12%)	0	4 (13%)	3 (30%)	4 (13%)	1 (10%)	20 (40%)	3 (30%)	13 (27%)	4 (40%)
Respiratory disease	10 (20%)	1 (11%)	4 (13%)	1 (10%)	3 (10%)	3 (30%)	3 (6%)	0	4 (8%)	0
Diabetes	2 (4%)	0	2 (7%)	2 (20%)	0	0	0	1 (10%)	3 (6%)	1 (10%)

Data are n (%) unless otherwise specified. BMI=body-mass index. *Included Hispanic-Columbian, Indian, Japanese, and White Irish/English.

Table: **Baseline characteristics of prime-boost participants included in the analysis**

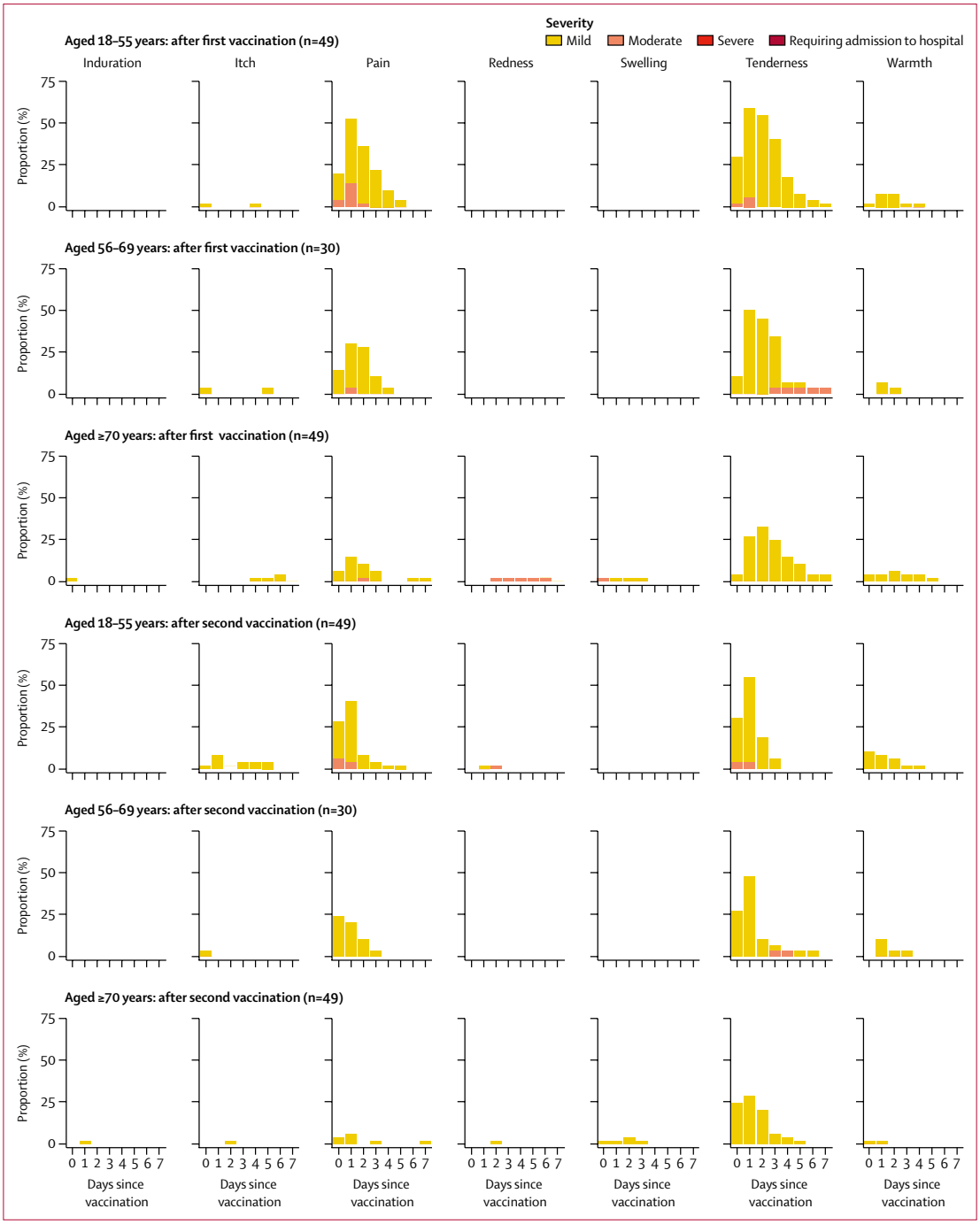


Figure 2: **Solicited local adverse reactions in the 7 days after prime and boost doses of standard-dose vaccine, by age**
Day 0 is the day of vaccination. Participants shown are those randomly assigned to receive two doses, and data are only shown for participants who received both doses of vaccine.

nCoV-19 was reported by 32 (65%) of 49 participants in the 18–55 years group, 21 (72%) of 29 in the 56–69 years group, and 21 (43%) of 49 in the 70 years and older group (appendix p 29). Within 7 days after the prime vaccination with ChAdOx1 nCoV-19, the incidence of objectively measured fever was low in the 18–55 years standard-dose

group (12 [24%] of 49), and no fevers were recorded in either the 56–69 years or 70 years and older standard-dose groups (appendix pp 16–18). No participants of any age who received the standard dose of ChAdOx1 nCoV-19 had objective fever after the boost vaccination. A similar pattern of decreasing reactogenicity with increasing age

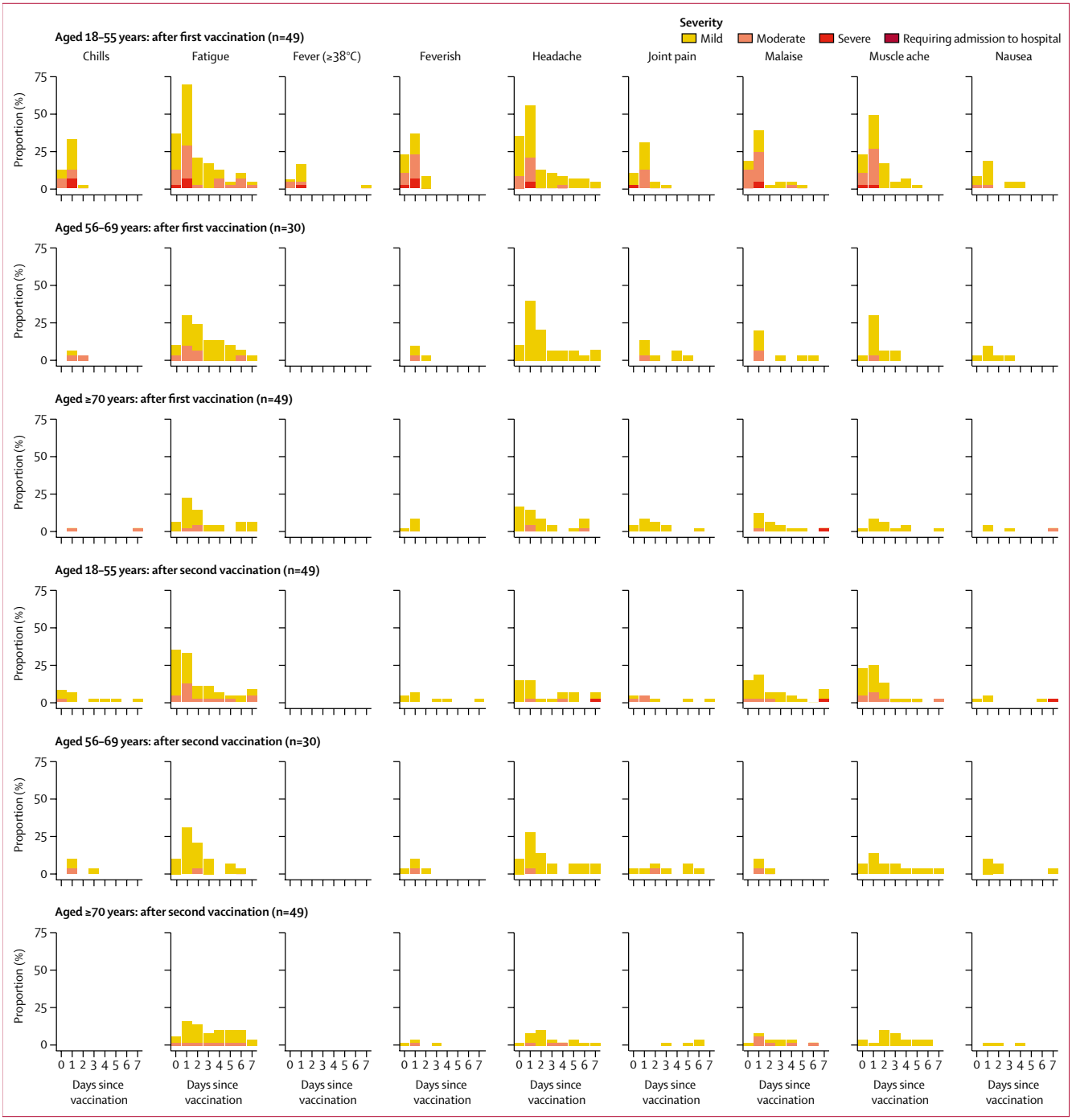


Figure 3: **Solicited systemic adverse reactions in the 7 days after prime and boost doses of standard-dose vaccine, by age**
Day 0 is the day of vaccination. Feverish is self-reported feeling of feverishness, whereas fever is an objective fever measurement (mild: 38·0 to <38·5°C, moderate: 38·5 to <39·0°C, severe: ≥39·0°C). Participants shown are those randomly assigned to receive two doses, and data are only shown for participants who received both doses of vaccine.

was seen in the low-dose groups (appendix pp 7, 8, 19–21). Similar results after the first dose were seen in those who were randomly assigned to receive only one dose of vaccine (data not shown). Data for the control groups are in the appendix (p 10).

As of Oct 26, 2020, 13 serious adverse events have occurred (across all age and vaccine groups), none of which are considered related to either study vaccine as assessed by the investigators (appendix p 31).

Using a multiplex immunoassay that detected total IgG against RBD and trimeric spike protein, we observed that participants who received the prime vaccination of standard-dose ChAdOx1 nCoV-19 had similar anti-spike antibody titres by day 28 after their prime vaccination as those who received a low dose (p=0·12 adjusted for age; figure 4; appendix p 12). At both dose levels, and for all dose groups combined, anti-spike IgG responses at day 28 decreased with increasing age (low-dose groups: 18–55 years, median 6439 arbitrary units [AU]/mL [IQR 4338–10 640], n=49; 56–69 years, 4553 AU/mL [2657–12 462], n=60; ≥70 years, 3565 AU/mL [1507–6345], n=93; p=0·0037; standard-dose groups: 18–55 years, median 9807 AU/mL [IQR 5847–17 220], n=43; 56–69 years, 5496 AU/mL [2548–12 061], n=55; ≥70 years, 4156 [2122–12 595], n=97; p=0·0044). By 28 days after the boost vaccination, similar antibody titres were seen across all two-dose groups, regardless of age or vaccine dose (eg, standard-dose groups: 18–55 years, median 20 713 AU/mL [IQR 13 898–33 550], n=39; 56–69 years, 16 170 AU/mL [10 233–40 353], n=26; and ≥70 years, 17 561 AU/mL [9705–37 796], n=47; p=0·68), and were higher than for those who did not receive a boost vaccination (appendix p 13). Similar results were seen with anti-RBD antibodies (figure 4; appendix p 12) and with an in-house standardised ELISA (appendix pp 12–13). Data for the control group are in the appendix (pp 12–13).

In a live SARS-CoV-2 microneutralisation assay (MNA_{80}), median titres peaked by day 42 in most groups that received two vaccinations (figure 5). There were no significant differences in normalised titres between age groups at day 42 (low-dose groups: 18–55 years, median 161 [IQR 99–233], n=41; 56–69 years, 143 [79–220], n=28; ≥70 years, 150 [103–255], n=34; p=0·90; standard-dose groups: 18–55 years, median 193 [IQR 113–238], n=39; 56–69 years, 144 [119–347], n=20; and ≥70 years, 161 [73–323], n=47; p=0·40). Within each age group, no significant differences were seen in neutralisation titres between low-dose and standard-dose vaccine recipients at the same timepoint (18–55 years p=0·33, 56–69 years p=0·12, ≥70 years p=0·62; figure 5; appendix p 14). Neutralising titres were achieved by 14 days after the boost vaccination in 208 (>99%) of 209 recipients of a boost vaccination. The one participant with a non-neutralising level was in the 70 years and older two-dose low-dose group.

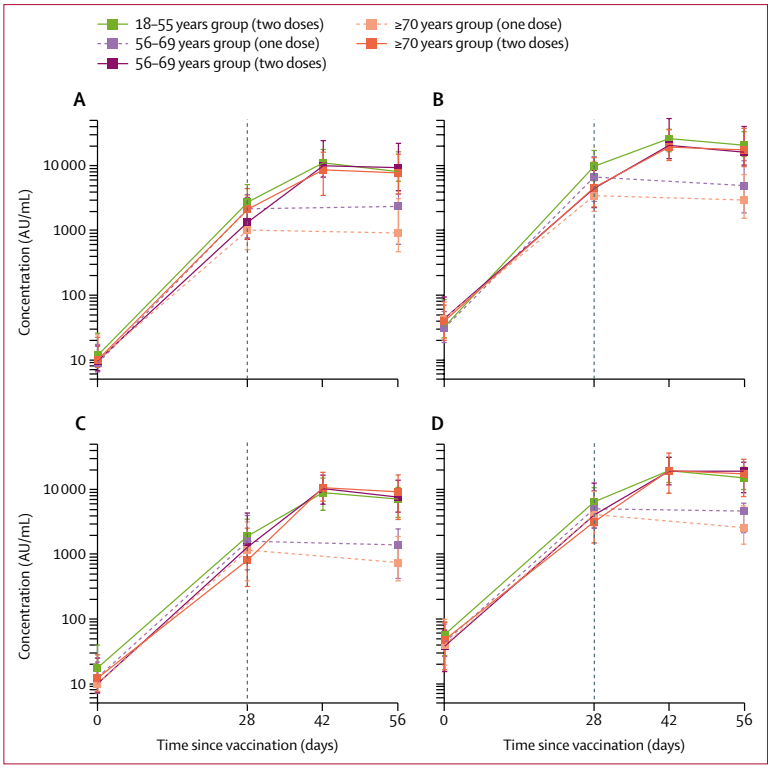


Figure 4: **SARS-CoV-2 IgG response to the receptor binding domain in the standard-dose groups (A) and low-dose groups (C) and the spike protein in the standard-dose groups (B) and the low-dose groups (D), by age**

Datapoints are medians, with whiskers showing the IQRs. Solid lines show participants who were randomly assigned to and received two doses of vaccine and dashed lines indicate participants who were randomly assigned to receive one dose. The vertical black line indicates when participants who received two doses received their boost dose. Data for the control groups are shown in the appendix (p 12). AU=arbitrary units. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

Anti-spike IgG levels after vaccination across all timepoints in those who received two doses of vaccine were highly correlated with neutralising titres in all age groups and for both low-dose and standard-dose vaccines (r^2 from linear regression 0·42–0·75, all p<0·0001; appendix p 32).

IFN-γ ELISpot responses against SARS-CoV-2 spike protein peaked 14 days after the prime vaccination (standard-dose groups: 18–55 years, median 1187 spot-forming cells [SFCs] per million peripheral blood mononuclear cells [PBMCs; IQR 841–2428], n=24; 56–69 years, 797 SFCs [383–1817], n=29; and ≥70 years, 977 SFCs [458–1914], n=48; appendix p 16) and did not increase significantly after the boost vaccination (p=0·46 from paired Student's *t* test of day 28 *vs* day 42; figure 6). ELISpot data were unavailable for the 18–55 years low-dose group because PBMCs were not collected in this group. In those who received two standard doses of vaccine, a significant difference was seen across age groups with those aged 56–69 years having higher responses at day 42 than other age groups receiving the

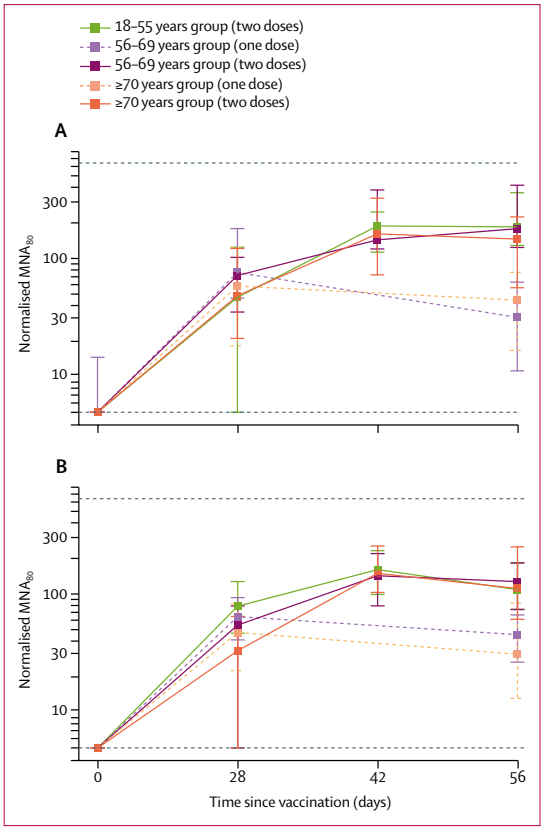


Figure 5: **Neutralising antibody titres measured using a live SARS-CoV-2 microneutralisation assay (MNA_{80}) after prime and boost doses of vaccine in standard-dose groups (A) and low-dose groups (B), by age**

Datapoints are medians, with whiskers showing the IQR. Solid lines show participants who were randomly assigned to and received two doses of vaccine and dashed lines indicate participants who were randomly assigned to receive one dose. Horizontal dotted lines show upper and lower limits of assay (values outside this range set to 640 beyond the upper limit and 5 beyond the lower limit). Data for the control groups are shown in the appendix (p 14).

To normalise data across assay runs, a reference sample was included in all assay runs and test samples normalised to this value by generating $\log_{10}$ ratios. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

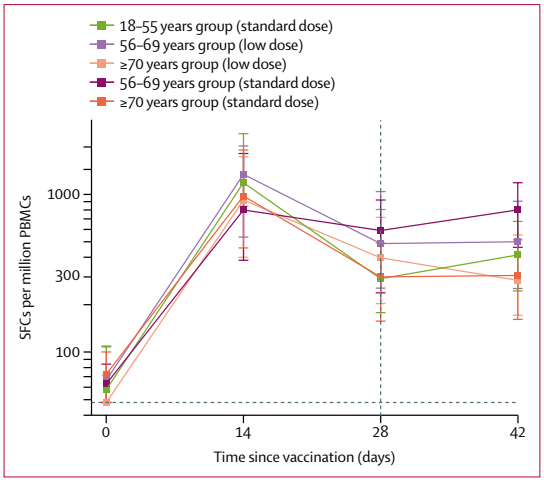


Figure 6: **IFN-γ ELISpot response to peptides spanning the SARS-CoV-2 spike insert after prime and boost doses of vaccine for all participants who were given two doses of vaccine, by age group and vaccine dose**

ELISpot data were unavailable for the 18–55 years low-dose group because PBMCs were not collected in this group. Datapoints are medians, with whiskers showing the IQR. The lower limit of detection is 48 SFCs per million PBMCs (horizontal dotted line). Day 42 samples are from participants who received the boost dose at day 28 (vertical dotted line). Data for both one-dose and two-dose groups, with numbers analysed at each timepoint, are in the appendix (p 15). ELISpot=enzyme-linked immunospot. PBMC=peripheral blood mononuclear cells. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. SFC=spot-forming cells.

same vaccine regimen (18–55 years, median 413 SFCs per million PBMCs [IQR 245–675], n=23; 56–69 years, 798 SFCs [462–1186], n=28; and ≥70 years, 307 SFCs [161–516], n=47; p<0·0001; appendix p 15).

Anti-ChAdOx1 neutralising antibody titres across different age and dose groups are shown in figure 7. Titres increased with the prime vaccination with ChAdOx1 nCoV-19 in all groups to similar levels, but were not increased further after a boost dose of vaccine at day 28. This observation was in contrast with the anti-SARS-CoV-2 spike protein antibody levels, which were increased 28 days after the boost vaccination (figure 4). Anti-ChAdOx1 neutralising titres immediately before the boost vaccination were negatively correlated with standardised ELISA values 28 days after the boost vaccination (p=0·037; figure 7), but no significant correlation was seen between anti-ChAdOx1 neutralising titres immediately before the boost vaccination and ELISpot responses 14 days after the boost vaccination (p=0·22; figure 7).

Discussion

Our findings show that the ChAdOx1 nCoV-19 vaccine was safe and well tolerated with a lower reactogenicity profile in older adults than in younger adults. Immunogenicity was similar across age groups after a boost vaccination. If these responses correlate with protection in humans, these findings are encouraging because older individuals are at disproportionate risk of severe COVID-19 and so any vaccine adopted for use against SARS-CoV-2 must be effective in older adults.

Most of the reported local and systemic adverse events were mild to moderate in severity, in line with our previous phase 1 study of the ChAdOx1 nCoV-19 vaccine[18] and previously reported studies of ChAdOx1-vectored vaccines.[22–24] Fewer adverse events were reported after the boost vaccination than after the prime vaccination and reactogenicity reduced with increasing age. The lower dose of vaccine was less reactogenic than the standard dose of vaccine across all age groups.

The serious adverse events observed during the trial in these study groups were judged to be unrelated to the study vaccines and occurred at frequencies expected for these conditions in the general population. None of the participants included in this report had any suspected

unexpected serious adverse reactions. In the phase 3 component of the trial, suspected unexpected serious adverse reactions occurred in other groups, and will be reported in detail in a subsequent publication. We carefully monitored suspected unexpected serious adverse reactions and other adverse events to ensure that no pattern of unexplained illnesses emerged that could indicate a safety concern. Independent assessments have led to the recommendation that the trial is safe to continue.

The ChAdOx1 nCoV-19 vaccine induced a specific antibody response to the SARS-CoV-2 spike glycoprotein and RBD at 28 days after a single dose across all age groups, including adults aged 70 years and older. A clear effect of a boost vaccination on antibody titres at day 56 was seen that was unrelated to dose regimen or age group. Similar patterns were observed with neutralising antibody responses, with no difference in the magnitude of the response at day 28 after the prime vaccine regardless of age or vaccine dose, but a booster effect was observed in individuals who received a second dose of vaccine.

Other clinical trials have also assessed safety, tolerability, and immunogenicity of SARS-CoV-2 vaccines in older adults. An adenovirus 5 vector-based vaccine also had reduced reactogenicity in adults aged 55 years and older compared with adults aged 18–54 years after a single dose of vaccine, although immunogenicity was concurrently reduced in this older age group.[11] A two-dose mRNA vaccine has also been shown to be immunogenic in adults older than 56 years with dose-dependent immune responses and similar neutralising antibody titres and cellular immune responses to younger adults.[9] Another two-dose mRNA vaccine has shown immunogenicity in older adults, but absolute neutralising antibody responses in adults aged 65–85 years were lower than in those aged 18–55 years.[10] By contrast with our observations, in both these studies, reactogenicity was more common after the second dose of an mRNA vaccine. A two-dose inactivated virus vaccine has also shown lower absolute neutralising antibody titres in adults aged 60 years and older than in adults aged 18–59 years, but reactogenicity was not formally compared between the first and second doses in this study.[13]

T-cell responses are important in controlling disease in natural infection[8] and therefore generation of a robust cellular immune response is a desirable attribute for a vaccine against SARS-CoV-2. Here, we found that spike-specific T-cell responses measured with ELISpot peaked at 14 days after the prime vaccination, consistent with previous studies of simian adenovirus-vectored vaccines,[25] and were similar in all groups regardless of age and vaccine dose. Spike protein T-cell responses measured with ELISpot have also been reported in studies with other adenovirus-vectored vaccines against SARS-CoV-2,[12] including in adults older than 55 years.[11] Theoretical concerns about vaccine-enhanced disease have led to a

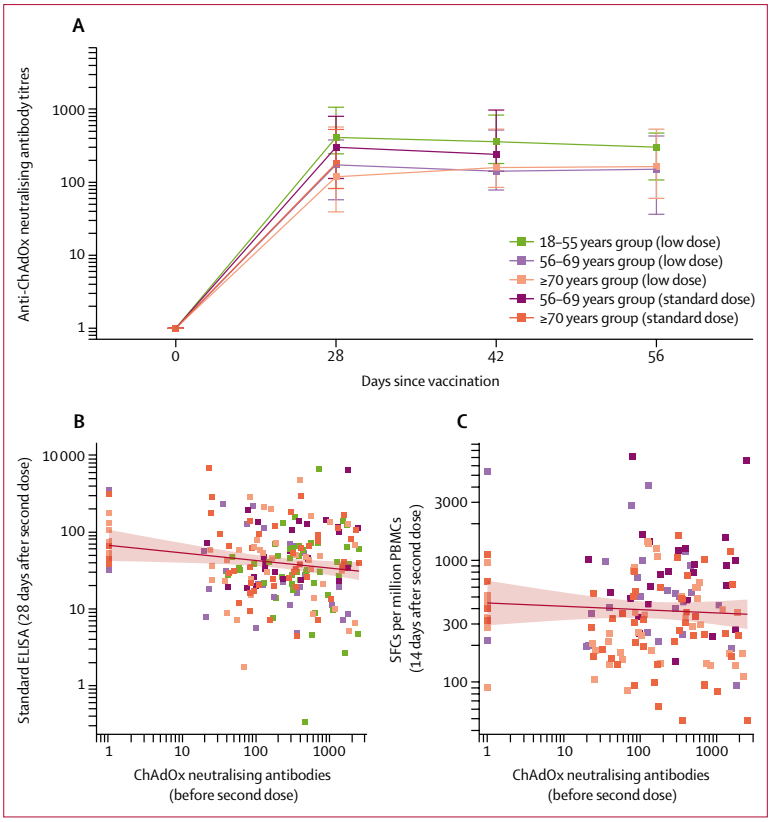


Figure 7: **Anti-ChAdOx1 vector neutralising titres after prime and boost doses of vaccine, by age and vaccine dose, and the correlation between pre-boost dose anti-ChAdOx1 neutralising antibodies and 28 days after boost dose antibody and T-cell responses**

(A) Anti-ChAdOx1 neutralising antibody titres in participants who received ChAdOx1 nCoV-19 vaccine by age and dose: datapoints are medians, with whiskers showing the IQR. Values below the limit of detection were assigned a value of 1. (B) Anti-ChAdOx1 neutralising antibody titre immediately before boost dose of vaccine versus standardised IgG ELISA against SARS-CoV-2 spike 28 days after the boost dose of vaccine with linear regression of logged values (p=0·037). (C) Anti-ChAdOx1 neutralising antibody titres immediately before boost dose of vaccine versus SARS-CoV-2 spike specific T cells measured by IFN-γ ELISpot on day 14 after the boost dose of vaccine with linear regression of logged values (p=0·22). In B and C, each datapoint is one participant and the solid line shows the linear regression, with the shaded area showing the 95% CI from an unadjusted linear regression of anti-vector neutralisation titres against logged ELISA (in B) or ELISpot (in C) response. Data were unavailable at day 56 for the 56–69 years standard-dose group. ELISpot=enzyme-linked immunospot. PBMC=peripheral blood mononuclear cells. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. SFC=spot-forming cells.

view that a type 1 T-helper (Th1)-biased CD4 response is a preferred coronavirus vaccine characteristic.[26] An adjuvanted nanoparticle vaccine has been shown to induce spike-specific CD4 T-cell cytokine responses with a predominantly Th1 profile,[15] as has an mRNA vaccine in small numbers of adults aged 56–70 years and 71 years and older.[9] More detailed investigations of antigen-specific T-cell responses in our study participants are ongoing.

The robust humoral and cellular immune responses obtained in our older adult population were encouraging given that a number of studies have shown that decreasing immune function with age leads to decreased immune responses to vaccines. This fact holds true for vaccines such as for influenza, for which pre-existing

immune memory exists,[27] and vaccines that induce primary immune responses, such as hepatitis B.[28] Other adenovirus-vector platforms against SARS-CoV-2 have either shown reduced immunogenicity in an older age group[11] (although this study was of a single-dose regimen and so not directly comparable with our prime-boost regimen) or have not yet been tested in an older population.[12]

However, our results are consistent with previous studies of adenovirus-vector-based vaccines against respiratory pathogens that evoke humoral and T-cell responses in older adults, including a human adenovirus-vectored respiratory syncytial virus (RSV) vaccine[29] and a simian adenovirus-vectored RSV vaccine.[30] Our results with ChAdOx1 nCoV-19 are also consistent with those of a ChAdOx1-vectored vaccine against influenza that showed good immunogenicity in adults older than 50 years.[22]

Notably, the anti-spike antibody responses in our study increased after a boost vaccination at an interval of 1 month but the neutralising anti-vector antibody responses did not. There was also no difference in anti-vector immunity by age. We observed a small negative correlation between anti-vector antibody titres and anti-spike total IgG, but not T-cell ELISpot responses. Further work is needed to investigate if homologous boosting with adenovirus-vectored vaccines can be done without loss of immunogenicity to the pathogen-specific transgene.

In the absence of a clear serological correlate of protection against SARS-CoV-2, clinical studies have focused on measuring neutralising antibodies because these have been shown to confer protection from challenge in animal models.[9–15] Live virus neutralisation assays are labour intensive and can only be done in specialist laboratories under category 3 biological safety conditions. We found here that anti-spike IgG levels correlate with neutralising antibody titres for all age groups. This finding suggests that, should neutralising antibodies be shown to be protective in humans, routine serological assays could be used for the standardised evaluation of functional antibody by vaccine candidates in clinical trials.

A limitation of this study is its single-blind design. However, all laboratory analyses and clinical assessments reported in this manuscript were done in a blinded fashion. A further limitation is possible variation of severity of local reactions due to the difference in injection volumes between different batches of vaccine in the low-dose group. Ongoing studies in larger groups will investigate the reactogenicity of a booster dose in more detail. Finally, the selection of participants aged 70 years and older, with a median age of 73–74 years between dose groups and with few comorbidities, might not be representative of the general older population, including those living in residential care settings or older than 80 years. Early phase studies in older adults require healthy volunteers to be enrolled for safety assessments, and recruitment to the study occurred during a period of national lockdown when more susceptible individuals were advised by Public Health England to self-isolate. Therefore, we excluded volunteers with substantial comorbidities or clinical frailty. Larger studies are now underway to assess immunogenicity, safety, and efficacy in older adults with a wider range of comorbidities.

Ultimately, licensure of a vaccine relies on the demonstration of efficacy in preventing COVID-19 and safety. Phase 3 studies with ChAdOx1 nCoV-19 are ongoing in the UK, Brazil, and the USA to assess vaccine efficacy and safety. Here we found similar safety and immunogenicity of ChAdOx1 nCoV-19 in older adults compared with younger adults, which could support the use of this vaccine in this older age group, if it is shown to be protective in phase 3 trials.

Contributors

AJP and SCG conceived and designed the trial and AJP is the chief investigator. AJP, AMM, HR, MNR, MV, and PMF contributed to the protocol and design of the study. AVSH and SNF were the study site principal investigators. ALF, CD, EAC, KJE, RM, and TL were responsible for laboratory testing and assay development. MV and NGM did the statistical analysis. SCG and TL were responsible for vaccine development. ADD, CG, and RT were responsible for vaccine manufacture. AJP, AMM, MNR, MV, NGM, and TL contributed to the preparation of the report. AMM, DRO, HR, KJE, MNR, PKA, and PMF contributed to the implementation of the study. All other authors contributed to the implementation of the study and data collection. All authors critically reviewed and approved the final version.

Declaration of interests

Oxford University has entered into a partnership with AstraZeneca for further development of ChAdOx1 nCoV-19 (AZD1222). AstraZeneca reviewed the data from the study and the final manuscript before submission, but the authors retained editorial control. SCG is cofounder of Vaccitech (a collaborator in the early development of this vaccine candidate) and named as an inventor on a patent covering use of ChAdOx1-vectored vaccines (PCT/GB2012/000467) and a patent application covering this SARS-CoV-2 vaccine. TL is named as an inventor on a patent application covering this SARS-CoV-2 vaccine and was consultant to Vaccitech. PMF is a consultant to Vaccitech. AJP is Chair of the UK Department of Health and Social Care's JCVI, but does not participate in policy advice on coronavirus vaccines, and is a member of the WHO Strategic Advisory Group of Experts (SAGE). AVSH is a cofounder of and consultant to Vaccitech and is named as an inventor on a patent covering design and use of ChAdOx1-vectored vaccines (PCT/GB2012/000467). MDS reports grants from Janssen, GlaxoSmithKline, MedImmune, Novavax, and MCM Vaccine and grants and non-financial support from Pfizer outside of the submitted work. CG reports personal fees from the Duke Human Vaccine Institute outside of the submitted work. ADD reports grants and personal fees from AstraZeneca outside of the submitted work. All other authors declare no competing interests.

Data sharing

The study protocol and clinical study plan are provided in the appendix (pp 45–212). Anonymised participant data will be made available when the trial is complete, upon requests directed to the corresponding author. Proposals will be reviewed and approved by the sponsor, investigator, and collaborators on the basis of scientific merit. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement. All data will be made available for a minimum of 5 years from the end of the trial.

Acknowledgments

This Article reports independent research funded by UK Research and Innovation (MC_PC_19055), Engineering and Physical Sciences Research Council (EP/R013756/1), Coalition for Epidemic Preparedness

Innovations, and the National Institute for Health Research (NIHR). We acknowledge support from Thames Valley and South Midland's NIHR Clinical Research Network and the staff and resources of NIHR Southampton Clinical Research Facility, and the NIHR Oxford Health Biomedical Research Centre. PMF received funding from the Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior, Brazil (finance code 001). ALF was supported by the Chinese Academy of Medical Sciences Innovation Fund for Medical Science, China (grant number: 2018-I2M-2-002). KJE is an NIHR Biomedical Research Centre Senior Research Fellow. AJP and SNF are NIHR senior investigators. The views expressed in this publication are those of the authors and not necessarily those of the NIHR or the UK Department of Health and Social Care. We thank the senior management at AstraZeneca for facilitating and funding the Mesoscale antibody assay included in this manuscript. We also thank the volunteers who participated in this study.

References

1 WHO. Coronavirus disease (COVID-19) dashboard. Geneva: World Health Organization, Nov 13, 2020. https://covid19.who.int (accessed Nov 13, 2020).

2 Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases From the Chinese Center for Disease Control and Prevention. *JAMA* 2020; **323:** 1239–42.

3 WHO. Draft landscape of COVID-19 vaccine candidates. Geneva: World Health Organization, Nov 12, 2020. https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines (accessed Nov 13, 2020).

4 WHO. WHO target product profiles for COVID-19 vaccines: version 3. Geneva: World Health Organization, April 29, 2020. https://www.who.int/docs/default-source/blue-print/who-target-product-profiles-for-covid-19-vaccines.pdf?sfvrsn=1d5da7ca_5 (accessed Oct 26, 2020).

5 Joint Committee on Vaccination and Immunisation. Priority groups for coronavirus (COVID-19) vaccination: advice from the JCVI, 25 September 2020. UK Government, Sept 25, 2020. https://www.gov.uk/government/publications/priority-groups-for-coronavirus-covid-19-vaccination-advice-from-the-jcvi-25-september-2020 (accessed Oct 26, 2020).

6 US Centers for Disease Control and Prevention. The COVID-19 vaccination program interim operational guidance for jurisdictions playbook. Nov 5, 2020. https://www.cdc.gov/vaccines/covid-19/covid19-vaccination-guidance.html (accessed Nov 16, 2020).

7 Aw D, Silva AB, Palmer DB. Immunosenescence: emerging challenges for an ageing population. *Immunology* 2007; **120:** 435–46.

8 Peng Y, Mentzer AJ, Liu G, et al. Broad and strong memory $CD4^+$ and $CD8^+$ T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. *Nat Immunol* 2020; **21:** 1336–45.

9 Anderson EJ, Rouphael NG, Widge AT, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. *N Engl J Med* 2020; published online Sept 29. https://doi.org/10.1056/NEJMoa2028436.

10 Walsh EE, Frenck RW Jr, Falsey AR, et al. Safety and immunogenicity of two RNA-based COVID-19 vaccine candidates. *N Engl J Med* 2020; published online Oct 14. https://doi.org/10.1056/NEJMoa2027906.

11 Zhu FC, Guan XH, Li YH, et al. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2020; **396:** 479–88.

12 Logunov DY, Dolzhikova IV, Zubkova OV, et al. Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. *Lancet* 2020; **396:** 887–97.

13 Xia S, Zhang Y, Wang Y, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. *Lancet Infect Dis* 2020; published online Oct 15. https://doi.org/10.1016/S1473-3099(20)30831-8.

14 Xia S, Duan K, Zhang Y, et al. Effect of an inactivated vaccine against SARS-CoV-2 on safety and immunogenicity outcomes: interim analysis of 2 randomized clinical trials. *JAMA* 2020; **324:** 951–60.

15 Keech C, Albert G, Cho I, et al. Phase 1-2 Trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. *N Engl J Med* 2020; published online Sept 2. https://doi.org/10.1056/NEJMoa2026920.

16 Milligan ID, Gibani MM, Sewell R, et al. Safety and immunogenicity of novel adenovirus type 26- and modified vaccinia Ankara-vectored Ebola vaccines: a randomized clinical trial. *JAMA* 2016; **315:** 1610–23.

17 van Doremalen N, Lambe T, Spencer A, et al. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. *Nature* 2020; **586:** 578–82.

18 Folegatti PM, Ewer KJ, Aley PK, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* 2020; **396:** 467–78.

19 Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ* 2005; **173:** 489–95.

20 Nimenrix: summary of medicinal product characteristics. Oct 28, 2020. https://www.medicines.org.uk/emc/medicine/26514#gref (accessed Oct 29, 2020).

21 Dicks MD, Spencer AJ, Edwards NJ, et al. A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. *PLoS One* 2012; **7:** e40385.

22 Coughlan L, Sridhar S, Payne R, et al. Heterologous two-dose vaccination with simian adenovirus and poxvirus vectors elicits long-lasting cellular immunity to influenza virus A in healthy adults. *EBioMedicine* 2018; **29:** 146–54.

23 Tapia MD, Sow SO, Mbaye KD, et al. Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in children in Africa: a randomised, observer-blind, placebo-controlled, phase 2 trial. *Lancet Infect Dis* 2020; **20:** 719–30.

24 Wilkie M, Satti I, Minhinnick A, et al. A phase I trial evaluating the safety and immunogenicity of a candidate tuberculosis vaccination regimen, ChAdOx1 85A prime - MVA85A boost in healthy UK adults. *Vaccine* 2020; **38:** 779–89.

25 Ewer K, Rampling T, Venkatraman N, et al. A monovalent chimpanzee adenovirus Ebola vaccine boosted with MVA. *N Engl J Med* 2016; **374:** 1635–46.

26 Lambert PH, Ambrosino DM, Andersen SR, et al. Consensus summary report for CEPI/BC March 12–13, 2020 meeting: assessment of risk of disease enhancement with COVID-19 vaccines. *Vaccine* 2020; **38:** 4783–91.

27 Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: a quantitative review. *Vaccine* 2006; **24:** 1159–69.

28 Denis F, Mounier M, Hessel L, et al. Hepatitis-B vaccination in the elderly. *J Infect Dis* 1984; **149:** 1019.

29 Williams K, Bastian AR, Feldman RA, et al. Phase 1 safety and immunogenicity study of a respiratory syncytial virus vaccine with an adenovirus 26 vector encoding prefusion F (Ad26.RSV.preF) in adults aged ≥60 years. *J Infect Dis* 2020; **222:** 979–88.

30 Green CA, Sande CJ, Scarselli E, et al. Novel genetically-modified chimpanzee adenovirus and MVA-vectored respiratory syncytial virus vaccine safely boosts humoral and cellular immunity in healthy older adults. *J Infect* 2019; **78:** 382–92.

Centers for Disease Control and Prevention

The Janssen COVID-19 Vaccine's Local Reactions, Systemic Reactions, Adverse Events, and Serious Adverse Events

Local Reactions

Local reactions were reported at higher rates by vaccine recipients than placebo recipients. The frequency of any local reaction was higher in participants aged 18 to 59 years than participants aged ≥60 years (59.8% vs 35.4%). Pain at the injection site was the most frequently reported solicited local reaction among vaccine recipients (58.6% of 18-59-year-olds and 33.3% ≥60-year-olds). Erythema and swelling were reported less frequently. No grade 4 local reactions were reported. Overall, the median onset of local reactions in the vaccine group was within two days of vaccination, with a median duration 2 days for erythema and pain and 3 days for swelling. (Table 1)

Table 1. Local reactions in persons aged 18-59 years and persons aged ≥60 years, Janssen COVID-19 vaccine and placebo[a]

	18-59 years		≥60 years	
	Janssen Vaccine N=2036	Placebo N=2049	Janssen Vaccine N=1320	Placebo N=1331
Any Local, n (%)				
Any	1218 (59.8)	413 (20.2)	467 (35.4)	244 (18.3)
Grade 3	18 (0.9)	4 (0.2)	5 (0.4)	2 (0.2)
Pain[b], n (%)				
Any	1193 (58.6)	357 (17.4)	439 (33.3)	207 (15.6)
Grade 3	8 (0.4)	0 (0.0)	3 (0.2)	2 (0.2)
Erythema[c], n (%)				
Any	184 (9.0)	89 (4.3)	61 (4.6)	42 (3.2)
Grade 3	6 (0.3)	2 (0.1)	1 (0.1)	0 (0.0)
Swelling[c], n (%)				
Any	142 (7.0)	32 (1.6)	36 (2.7)	21 (1.6)
Grade 3	5 (0.2)	2 (0.1)	2 (0.2)	0 (0.0)

[a] Solicited local and systemic adverse reactions collected for participants in a safety subset (N=6,736)

[b] Pain – Grade 3: any use of prescription pain reliever or prevented daily activity

[c] Erythema and Swelling – Grade 3: >100mm

Note: No grade 4 local reactions were reported.

Systemic Reactions

Systemic reactions were reported at higher rates by vaccine recipients than placebo recipients. The frequency of systemic reactions was higher in participants aged 18-59 years than participants ≥60 years (61.5% vs 45.3%). For both age groups, fatigue and headache were the most commonly reported systemic reactions. Fever was more common in participants 18-59

years (12.8%) compared to those ≥60 years (3.1%). The majority of systemic reactions were mild or moderate in severity. The most common grade 3 reactions were fatigue and myalgia. No grade 4 reactions were reported. Among vaccine recipients, the median onset of systemic reactions within 2 days of vaccination, with a median duration of 1-2 days. (Table 2)

Table 2. Systemic reactions in persons aged 18-59 years and persons aged ≥60 years, Janssen COVID-19 vaccine and placebo[a]

	18-59 years		≥60 years	
	Janssen Vaccine N=2036	Placebo N=2049	Janssen Vaccine N=1320	Placebo N=1331
Any systemic, n (%)				
Any	1252 (61.5)	745 (36.4)	598 (45.3)	440 (33.1)
Grade 3	47 (2.3)	12 (0.6)	14 (1.1)	9 (0.7)
Fatigue[b], n (%)				
Any	891 (43.8)	451 (22.0)	392 (29.7)	277 (20.8)
Grade 3	25 (1.2)	4 (0.2)	10 (0.8)	5 (0.4)
Headache[b], n (%)				
Any	905 (44.4)	508 (24.8)	401 (30.4)	294 (22.1)
Grade 3	18 (0.9)	5 (0.2)	5 (0.4)	4 (0.3)
Myalgia[b], n (%)				
Any	796 (39.1)	248 (12.1)	317 (24.0)	182 (13.7)
Grade 3	29 (1.4)	1 (<0.1)	3 (0.2)	5 (0.4)
Nausea[c], n (%)				
Any	315 (15.5)	183 (8.9)	162 (12.3)	144 (10.8)
Grade 3	3 (0.1)	3 (0.1)	3 (0.2)	3 (0.2)
Fever[d], n (%)				
Any	261 (12.8)	14 (0.7)	41 (3.1)	6 (0.5)
Grade 3	7 (0.3)	0 (0.0)	1 (0.1)	0 (0.0)

[a] Solicited local and systemic adverse reactions collected for participants in a safety subset (N=6,736)

[b] Fatigue, Headache, Myalgia – Grade 3: use of prescription pain reliever or prevented daily activity

[c] Nausea – Grade 3: prevented daily activity

[d] Fever – Grade 3: ≥39.0 – ≤40.0°C or ≥102.1 – ≤104.0°F

Note: No grade 4 systemic reactions were reported.

Analgesic/Antipyretics Use

Among vaccine recipients aged 18-59 years, 26.4% reported using antipyretic or analgesic medications, compared to 6.0% of placebo recipients. Among vaccine recipients aged ≥60 years, 9.8% reported using antipyretic or analgesic medications, compared to 5.1% of placebo recipients. The reason for medication use (e.g. fever, pain) was not ascertained.

Unsolicited Adverse Events

Overall, rates of reported unsolicited adverse events were similar in the vaccine and placebo groups (13.1% vs 12.0%). Reports of embolic and thrombotic events had a slight numerical imbalance with 0.06% of vaccine recipients and 0.05% of placebo recipients reporting such events. Risk factors for these events were present in the participants, however vaccine cannot be excluded as a contributing factor. Reports of tinnitus had a numerical imbalance with 6 events in vaccine recipients and no events in placebo recipients. Data are insufficient at this time to determine if there is a casual relationship between the

vaccine and tinnitus. Angioedema demonstrated a numerical imbalance with events reported among 0.2% of vaccine recipients and 0.1% of placebo recipients. Of these, urticaria was reported in 8 vaccine recipients and 3 placebo recipients. Based on temporal and biologic plausibility, reports of urticaria are possibly related to vaccine.

Serious Adverse Events

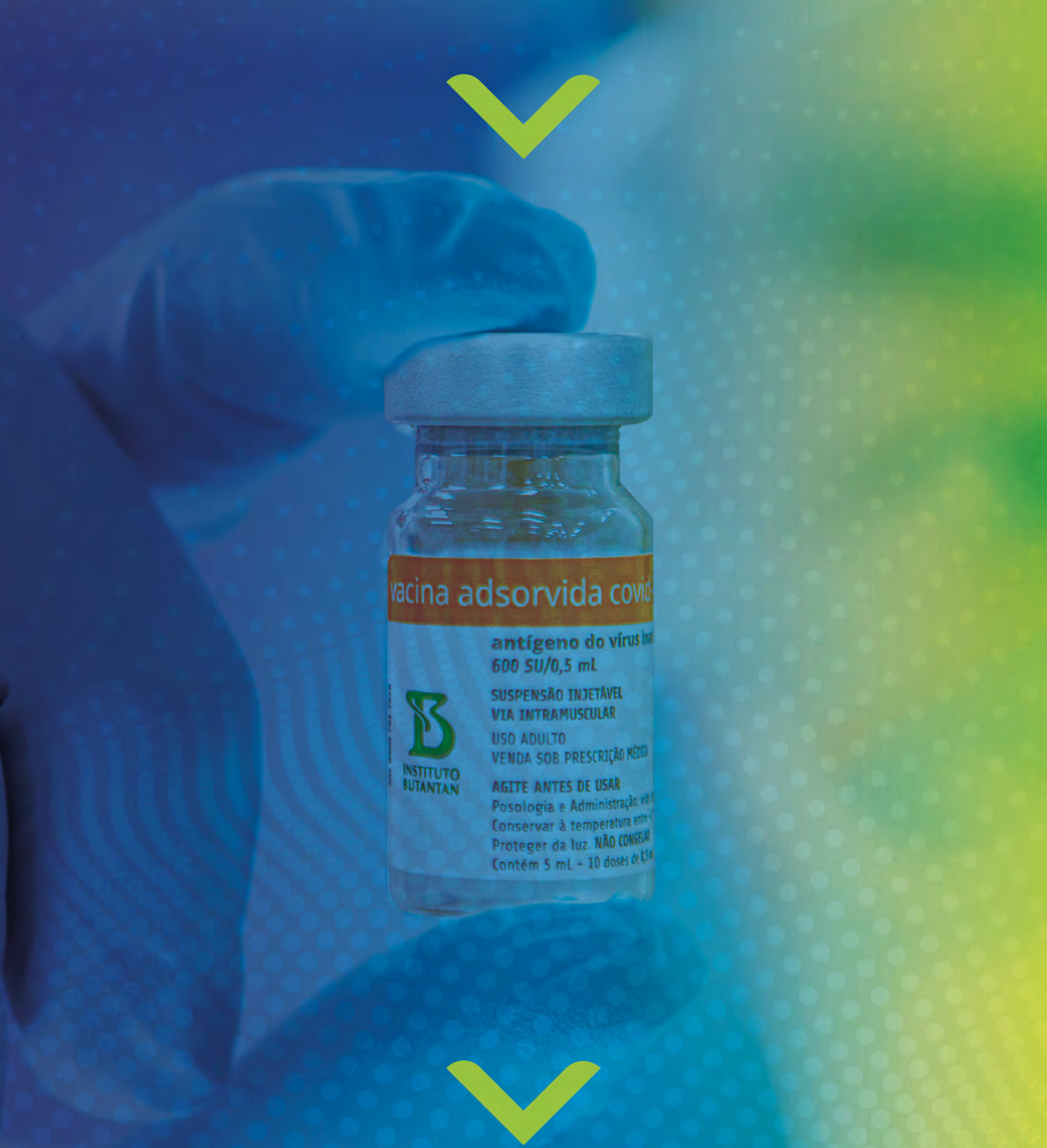
Serious adverse events were defined as any untoward medical occurrence that resulted in death, was life-threatening, required inpatient hospitalization or prolongation of existing hospitalization, or resulted in persistent disability or incapacity. The proportions of participants who reported at least one serious adverse event, excluding those attributed to COVID-19, were 0.4% in the vaccine group and 0.4% in the placebo group. The most common serious adverse event occurring at higher rates in the vaccine group than the placebo group was appendicitis (6 cases in vaccine group vs. 5 cases in placebo group). Three serious adverse events occurring among vaccine recipients were considered by the U.S. Food and Drug Administration (FDA) as likely related to vaccine: the one report of hypersensitivity reaction to study vaccine, one report of pain at the injection site initially evaluated for brachial neuritis, and one report of systemic reactogenicity.

Data source: FDA briefing document

Page last reviewed: August 12, 2021

CoronaVac

O que a ciência comprova



Versão: 10/2022

fundação butantan

INSTITUTO BUTANTAN
A serviço da vida

GOVERNO DO ESTADO DE SÃO PAULO