



CoronaVac

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1. Highly immunogenic

1.1. CoronaVac offers 80% protection against hospitalizations and death in people reinfected with SARS-CoV-2, study shows

A real-world study carried out in Brazil with about 20,000 people proved once again the effectiveness of CoronaVac against severe COVID-19 cases and hospitalizations. The analysis showed that more than 80% of individuals reinfected with SARS-CoV-2 who had taken CoronaVac recovered without the need for hospitalization. Published in *The Lancet Infectious Diseases*, the work was carried out by scientists from Brazil and abroad, from institutions such as the University of São Paulo and Instituto Oswaldo Cruz (Fiocruz), as well as American universities such as Yale and Stanford.

Between February 2020 and November 2021, 213,000 Brazilians (vaccinated and not vaccinated) were identified as having symptomatic Covid-19 after the vaccination program started. To compare the effectiveness of CoronaVac, AstraZeneca, Pfizer and Janssen, scientists selected 22,500 cases from across the country who tested positive for SARS-CoV-2 reinfection, of which 1,545 ended up being hospitalized and 290 died.

Of the 22,500 cases analyzed, 8,000 were immunized – 42.8% had taken CoronaVac, 40% received AstraZeneca,

14.9% Pfizer and 2.2% Janssen. Among those vaccinated with CoronaVac, the effectiveness against hospitalization and death was 81.3%. The percentage was similar to the AstraZeneca (89.9%) and Pfizer (89.7%) vaccines, and higher than the results of Janssen (57.7%). It is worth mentioning that immunization with CoronaVac in Brazil started first, involving, in large majority, elderly people over 60 years old, a group more vulnerable to the worsening of Covid-19 cases.

According to the researchers, the analyses focused on individuals who were previously infected with the coronavirus to answer whether and to what extent vaccines provide additional protection against symptomatic infection and serious outcomes.

“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. We found that two doses of CoronaVac provided high levels of protection against severe outcomes”, the scientists in the article state.

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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

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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.

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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 infection⁵ and, if so, whether a single dose is sufficient.^{6,7} The emergence of more transmissible variants with enhanced immune

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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

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

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.

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

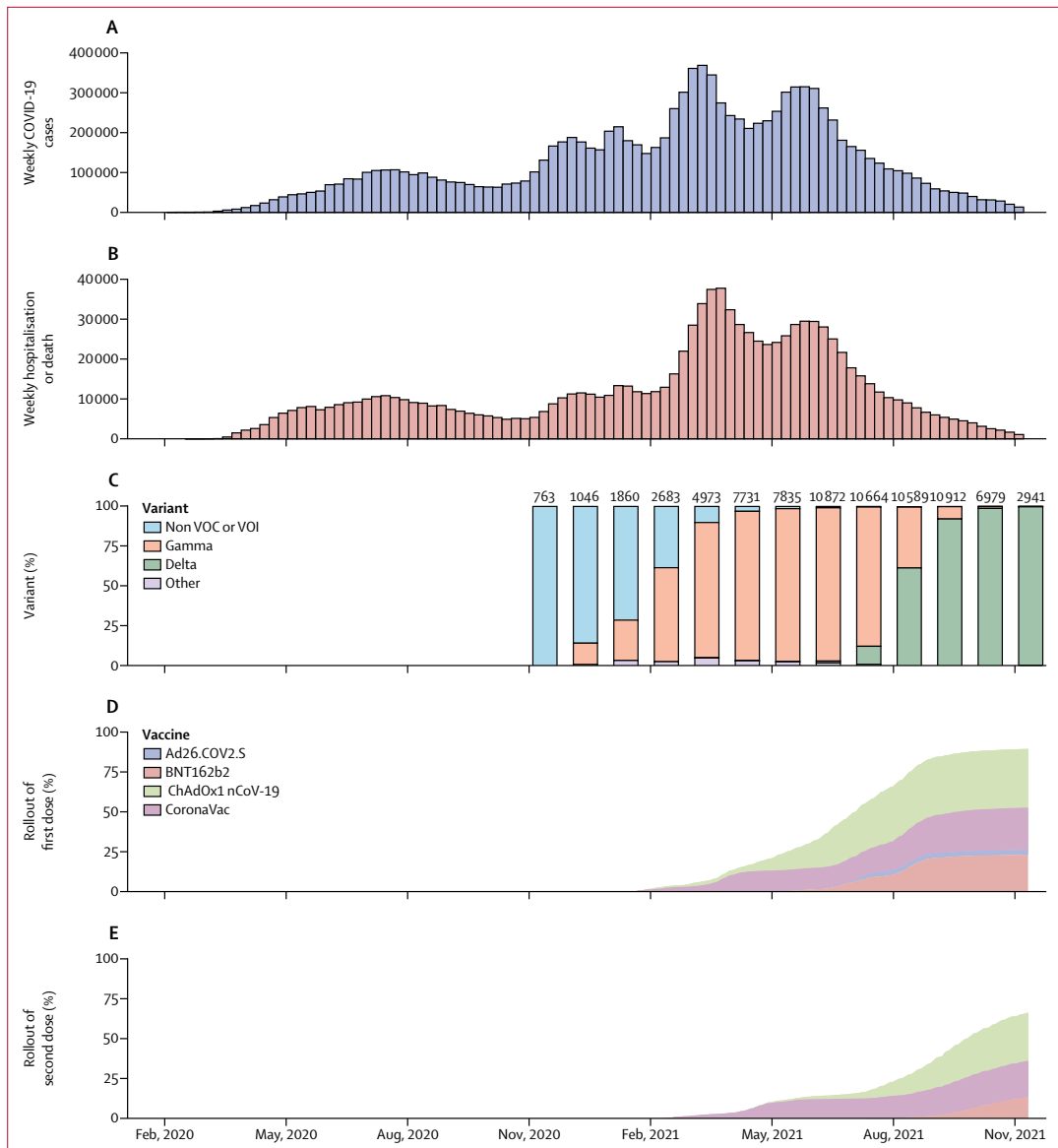


Figure 1: Temporal trends in COVID-19 cases, hospitalisation or deaths, variants, and vaccination coverage from national databases in Brazil
 Weekly numbers of symptomatic COVID-19 cases (A); COVID-19-associated hospitalisations or deaths reported in national databases (B); monthly proportions of variants 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 immunosuppression, 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

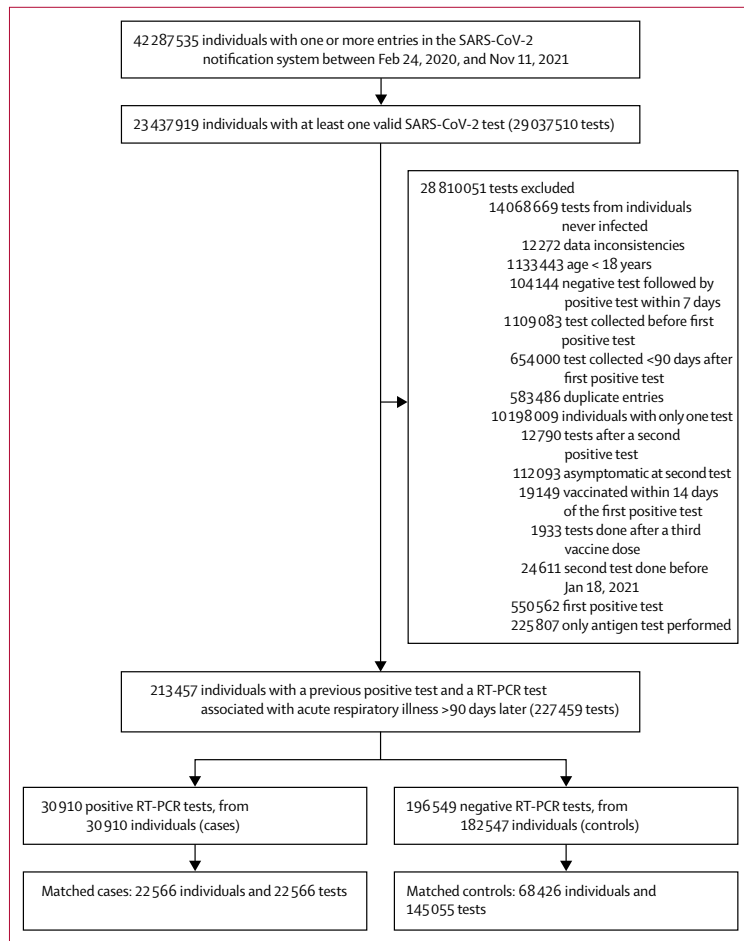


Figure 2: Flowchart of the study population from surveillance databases and selection of matched cases and controls

Cases and controls were matched on age (± 5 years), sex, municipality, and date of test (± 10 days).

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).

See Online for appendix

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

	Eligible population		Matched sets		Standardised difference
	Cases	Controls	Cases	Controls	
Individuals	30910	182547	22566	68426	..
Tests	30910	196549	22566	145055	..
Age, years	38 (29–47)	37 (28–47)	37 (29–46)	36 (29–44)	0.066
Sex	0.047
Female	18106 (58.6%)	119134 (60.6%)	13631 (60.4%)	90931 (62.7%)	..
Male	12804 (41.4%)	77415 (39.4%)	8935 (39.6%)	54124 (37.3%)	..
Race	0.039
White	13841 (44.8%)	109923 (55.9%)	10302 (45.7%)	67403 (46.5%)	..
Mixed	11363 (36.8%)	53401 (27.2%)	7998 (35.4%)	50788 (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%)	14886 (7.6%)	1777 (7.9%)	10541 (7.3%)	..
Region of residence	0.085
Central west	3260 (10.5%)	46968 (23.9%)	2302 (10.2%)	12997 (9.0%)	..
North	2406 (7.8%)	9724 (4.9%)	1870 (8.3%)	12372 (8.5%)	..
Northeast	8268 (26.7%)	30027 (15.3%)	5297 (23.5%)	30489 (21.0%)	..
South	2823 (9.1%)	16251 (8.3%)	1991 (8.8%)	14745 (10.2%)	..
Southeast	14153 (45.8%)	93579 (47.6%)	11106 (49.2%)	74452 (51.3%)	..
Residence in state capital	9250 (29.9%)	51128 (26.0%)	8982 (39.8%)	77198 (53.2%)	0.271
Medical comorbidities
None	25988 (84.1%)	166655 (84.8%)	19271 (85.4%)	124964 (86.1%)	0.027
One	3552 (11.5%)	22178 (11.3%)	2459 (10.9%)	15360 (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.COVS.2.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

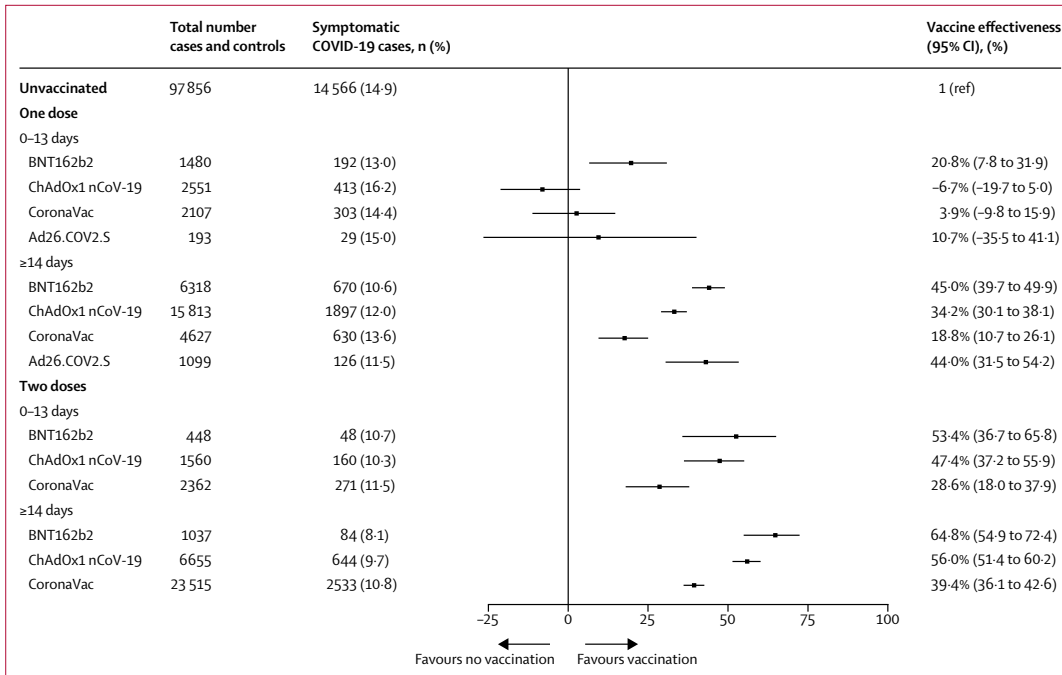


Figure 3: Effectiveness of BNT162b2, ChAdOx1 nCoV-19, CoronaVac, and Ad26.COVS.2.S vaccines against symptomatic COVID-19 among individuals with previous SARS-CoV-2 infection

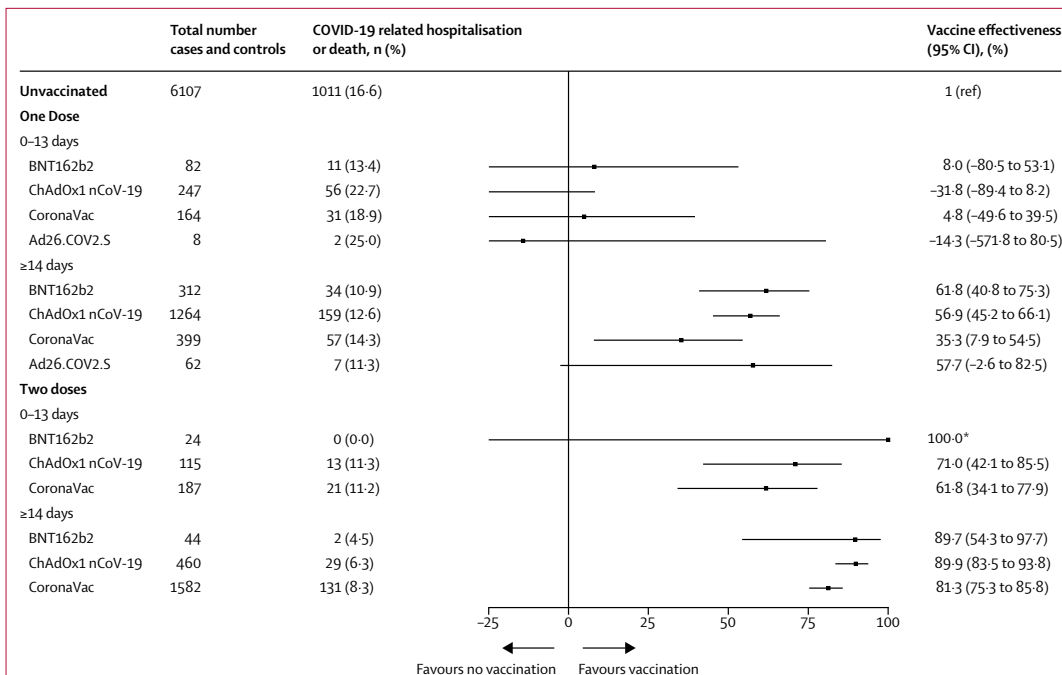


Figure 4: Effectiveness of BNT162b2, ChAdOx1 nCoV-19, CoronaVac, and Ad26.COVS.2.S vaccines against COVID-19-associated hospitalisation or death among individuals with previous SARS-CoV-2 infection

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

for BNT162b2 (35·3% vs 70·7%, $p=0\cdot011$; 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%; $p=0\cdot007$), whereas effectiveness was lower for CoronaVac at more than 90 days than at 14–90 days (74·4% vs 86·6%, $p=0\cdot012$; 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 became 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.

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1.2. CoronaVac multiplies neutralizing antibody levels in people recovered from Covid-19, Chilean study shows

A Chilean study published in The Lancet Discovery Science journal eBioMedicine showed that CoronaVac increases the geometric mean titers (GMT) of neutralizing antibodies more than threefold, to above 2,000, in individuals with previous SARS-CoV-2 infection. The research was conducted by the Pontifical Catholic University of Chile and Fundación Ciencia & Vida, among other institutions.

In subjects who had mild to moderate disease, CoronaVac raised the level of neutralizing antibodies from 174 to 2057.3 GMT. In those who had been hospitalized with severe disease, and therefore had more antibodies to fight the infection, the increase was from 700.8 to 2113.6 GMT.

To conduct this analysis, the scientists evaluated the natural immune response of 74 individuals recovering from Covid-19 and observed that there was a rapid drop in neutralizing antibodies (nAb) over 12 months. Of these participants, 30 were subsequently vaccinated with CoronaVac and most showed an increase in nAb titer, showing that the vaccine is effective in activating memory B cells, which produce antibodies.

In addition, the real-life effectiveness of CoronaVac in Chile was greater than the estimated efficacy in the study. While the estimated protection against Covid-19 infections was 50%, the actual protection

was 65.9%. The vaccine also protected 87.5% against hospitalizations, 90.3% against admissions in Intensive Care Units (ICU) and 86.3% against death. “The protection afforded by CoronaVac is most likely due to the induction of nAbs responses, and additional immune mechanisms, such as T-cell immunity and immune memory” the authors state.

According to the researchers, the drop in neutralizing antibodies over time in convalescent individuals reinforces that vaccination is necessary to potentiate the antibody response and memory cells. “Booster immunization strategies might be required to control the pandemic and prevent re-infection with new variants,” they point out.

CoronaVac increases protection against variants

A recently published research from China also showed that CoronaVac potentiates the immunity of previously infected individuals, inducing high neutralizing activity against the delta, alpha and beta variants of the SARS-CoV-2 virus, as well as increasing antibody amounts. Neutralizing antibodies against the original strain and its variants increased from seven to 17-fold.

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

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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 naïve 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 naïve 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%.

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Interpretation The decay of nAbs titres in previously infected individuals over time indicates that vaccination is needed to boost humoral memory responses. Immunization of naïve 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 BNT162b2. 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 BNT162b2 vaccine in naïve 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.

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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 vaccination⁷ 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 response¹⁵ 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 naïve 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 ($n = 35$; 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

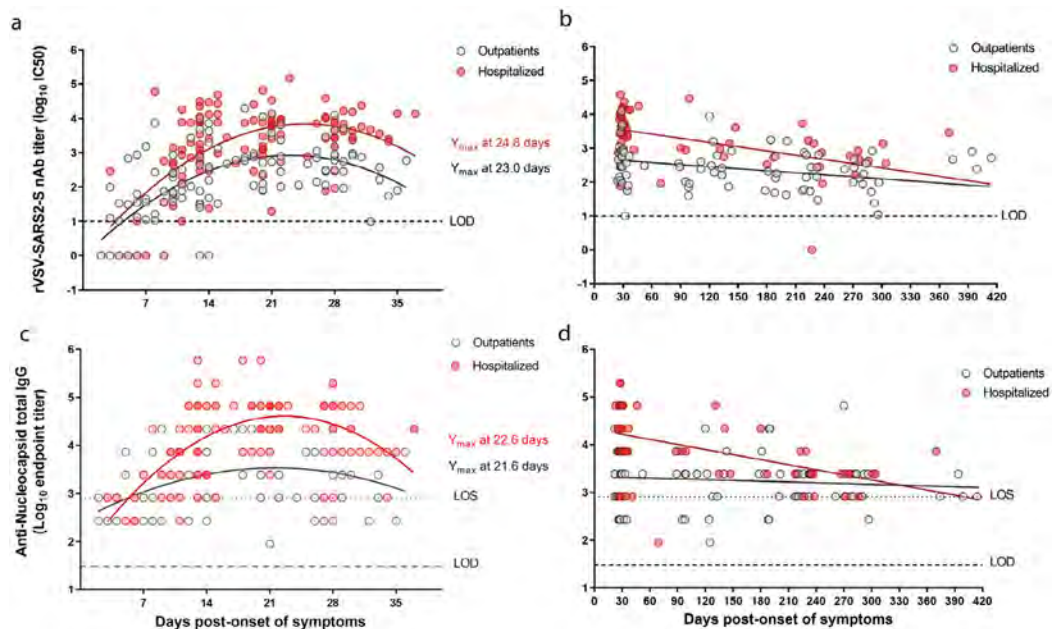


Figure 1. Longitudinal dynamics of neutralizing and anti-N antibody responses to SARS-CoV-2 infection from outpatient and hospitalized individuals. **a,b.** The half-maximum inhibitory concentration (IC₅₀) of sera was determined by microneutralization assay of recombinant vesicular stomatitis virus carrying 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 (Y_{max}). **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 naïve 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.

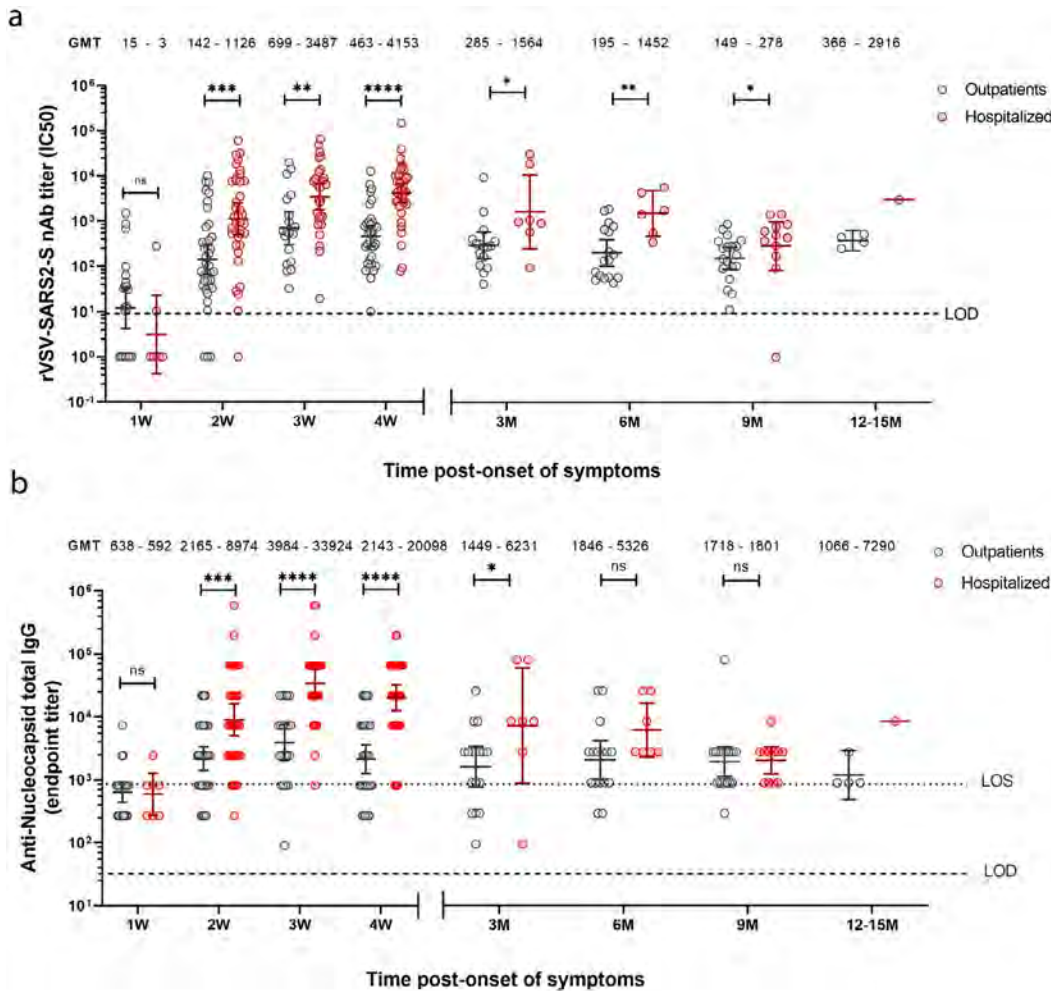


Figure 2. Comparison of neutralizing and anti-N antibody responses after SARS-CoV-2 infection of outpatient and hospitalized individuals over a 12 months period. **a.** nAb IC50 titres were determined by microneutralization assay of recombinant vesicular stomatitis virus carrying SARS-CoV-2 spike protein (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 ($*P < 0.05$; $**P < 0.01$; $**P < 0.001$; $****P < 0.0001$; 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-

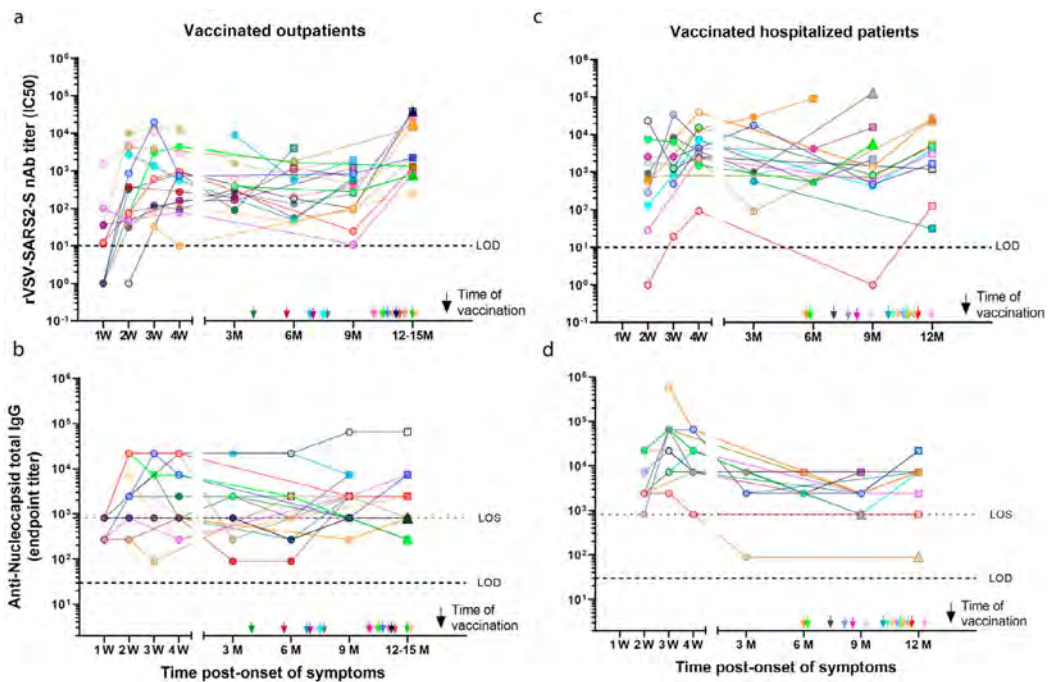


Figure 3. Longitudinal neutralizing and anti-N antibody titres to SARS-CoV-2 in previously infected before and after CoronaVac or BNT162b2 vaccination. nAb titres (IC50) obtained using a rVSV-SARS2-S microneutralization assay and end-point titres of anti-N IgG were determined by ELISA using a recombinant 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-

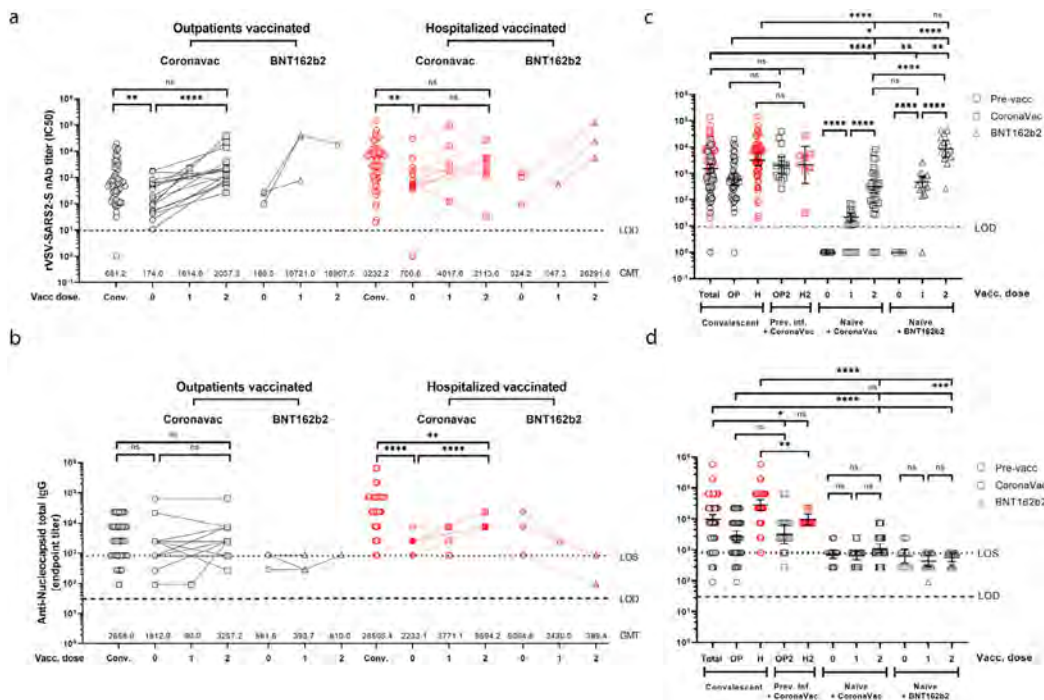


Figure 4. Neutralizing and anti-N antibody titres to SARS-CoV-2 in previously infected and naïve individuals before and after CoronaVac or BNT162b2 vaccination. nAb (b) and anti-N IgG (c) titres from 20 outpatient (42 samples) or 16 hospitalized (33 samples) individuals immunized with one or two doses of 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 (** $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; 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 # AB1010901000) 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 SIGMAFAST 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% CO₂ 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 confounding 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 longitudinal 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 confounding 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 naïve (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 naïve 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. Naïve 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 naïve 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 naïve individuals did not respond to immunization, we assessed for potential confounding

factors affecting vaccine response. There were no demographic or clinical variables associated with either seroconversion or lack of antibody induction in the naïve immunized group. We further analyzed all the vaccinee data, by including the previously infected and naïve-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 naïve 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 naïve 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 BNT162b2 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 naïve vaccinated individuals. While the overall demographics of this group was highly similar to the previously infected immunized group, the average age of the naïve 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 U19AI135972: Fluomics: The Next Generation.

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Supplementary materials

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

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CoronaVac

What science proves

1.3. CoronaVac-induced immune response is superior to natural infection, Turkey study shows

A study from Turkey published in the journal *Vaccine* once again showed that, in people who have never had Covid-19, CoronaVac induces greater protection than that observed in those who only had the infection and were not vaccinated. In addition, individuals with previous infection who take CoronaVac multiply their antibody amount. The study reinforces the importance of immunization and adds to other studies that have already shown that natural infection is not enough to produce a lasting immune response, since the vaccine induces protection superior to that caused by the disease alone.

Conducted at the School of Medicine of Marmara University in Istanbul, the survey included 224 healthcare professionals, divided into four groups: 75 volunteers with no history of Covid-19 who were vaccinated with CoronaVac; 53 who were immunized after having the infection; 60 with previous infection who were not vaccinated; and 36 who had Covid-19 after vaccination.

All study participants showed seroconversion (antibody production), with IgG antibody levels being higher in those who received the vaccine after having the disease (620 BAU/ml) and in those vaccinated without previous infection (136 BAU/ml), compared to those who only had the infection and were not immunized.

The group with the highest antibody production was that of those who had Covid-19 after vaccination, with 6,146 BAU/ml, demonstrating that the vaccine activates immunological memory and enhances the body's response against the infection. However, these data were not statistically compared because

the interval between the second dose of vaccine and blood sampling did not match the intervals of the other groups.

“Healthcare workers 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”, the authors point out.

The superiority of vaccines over natural infections is amply proven by science. A recent study conducted in China showed that people who took CoronaVac and were infected by the delta variant are protected against omicron, unlike those who had the disease and did not get vaccinated. Other research conducted in Turkey also showed that previously infected individuals who have been vaccinated with CoronaVac have increased antibody levels.

Only vaccination protects against hospitalization and death

As an inactivated virus vaccine, CoronaVac “mimics” the infection without causing the disease. And as effectiveness studies carried out around the world have proven, CoronaVac protects more than 90% against hospitalization and death. Learn more about the benefits of the Butantan vaccine in the CoronaVac Dossier.

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RESEARCH PAPER

Antibody response with SARS-CoV-2 inactivated vaccine (CoronaVac) in Turkish geriatric population

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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.

A. Okyar, Baş et al.

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

Antibody response with SARS-CoV-2

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. ^aP-value < 0.05 for the comparison between Group 1, 2 and 3. ^bP value < 0.05 for the comparison between Group 1 and 2. ^cP value < 0.05 for the comparison between Group 1 and 3. ^dP 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 EWGOP 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

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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 \pm 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

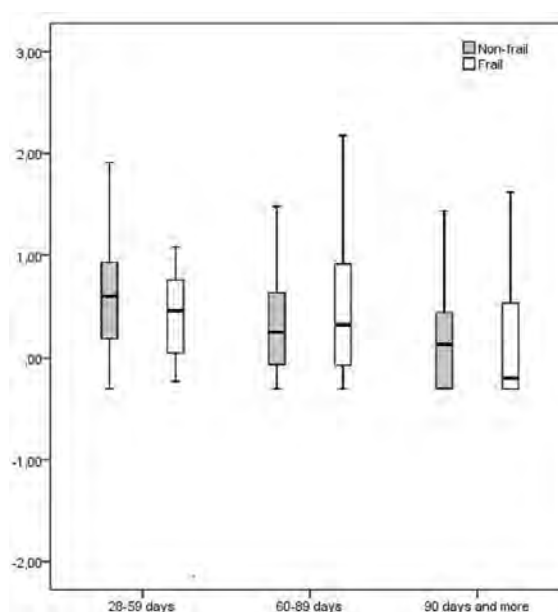


Figure 1. Distribution of antibody titres according to frailty status.

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 drawing 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

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Table 2. Comparison of seropositive and seronegative individuals in Group 1

	Sars-CoV2 spike IgG antibody positive group <i>n</i> = 152 (80.9%)	Sars-CoV2 spike IgG antibody negative group <i>n</i> = 36 (19.1%)	<i>P</i> value
Age, median (IQR)	71 (67–76)	72 (68–80.75)	0.272
Female gender <i>n</i> (%)	87 (58%)	22 (61.1%)	0.734
Comorbidities			
Depression, <i>n</i> (%)	16 (10.2%)	2 (5.6%)	0.533
CVD, <i>n</i> (%)	40 (26.7%)	9 (25.0%)	0.838
HT, <i>n</i> (%)	107 (71.3%)	28 (77.8%)	0.436
DM, <i>n</i> (%)	65 (43.3%)	19 (52.8%)	0.307
Atrial fibrillation, <i>n</i> (%)	13 (8.7%)	3 (8.3%)	0.949
Hypothyroidism, <i>n</i> (%)	27 (18.0%)	7 (19.4%)	0.840
Congestive heart failure, <i>n</i> (%)	13 (8.7%)	6 (16.7%)	0.215
Rheumatological diseases, <i>n</i> (%)	13 (8.7%)	7 (19.4%)	0.074
Malignancy history, <i>n</i> (%)	18 (12.0%)	7 (19.4%)	0.276
Chronic renal disease, <i>n</i> (%)	6 (4.0%)	2 (5.6%)	0.653
Pulmonary diseases, <i>n</i> (%)	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, <i>n</i> (%)	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, <i>n</i> (%)	88 (59.9%)	22 (61.1%)	0.891
Falls, <i>n</i> (%)	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, <i>n</i> (%)	45 (35.7%)	15 (51.7%)	0.111
Low gait speed, <i>n</i> (%)	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 <i>n</i> = 101 (73.2%)	Sars-CoV2 spike IgG antibody negative group <i>n</i> = 37 (26.7%)	<i>P</i> 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, <i>n</i> (%)	15 (15.3%)	4 (11.1%)	0.537
CVD, <i>n</i> (%)	20 (20.4%)	10 (27.8%)	0.364
HT, <i>n</i> (%)	76 (77.6%)	25 (69.4%)	0.334
DM, <i>n</i> (%)	38 (38.8%)	15 (41.7%)	0.742
Atrial fibrillation, <i>n</i> (%)	6 (6.1%)	5 (13.9%)	0.165
Hypothyroidism, <i>n</i> (%)	24 (24.5%)	4 (11.1%)	0.091
Congestive heart failure, <i>n</i> (%)	7 (7.1%)	3 (8.3%)	0.728
Rheumatological diseases, <i>n</i> (%)	2 (2.0%)	7 (19.4%)	0.001 ^{*,**}
Malignancy history, <i>n</i> (%)	7 (7.1%)	7 (19.4%)	0.055
Chronic renal disease, <i>n</i> (%)	6 (6.1%)	8 (3.8%)	0.701
Pulmonary diseases, <i>n</i> (%)	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, <i>n</i> (%)	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, <i>n</i> (%)	20 (55.6%)	55 (56.7%)	0.816
Falls, <i>n</i> (%)	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, <i>n</i> (%)	25 (30.9%)	7 (23.3%)	0.437
Low gait speed, <i>n</i> (%)	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

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Table 4. Comparison of seropositive and seronegative individuals, in Group 3

	Sars-CoV2 spike IgG antibody positive group <i>n</i> = 98 (57.3%)	Sars-CoV2 spike IgG antibody negative group <i>n</i> = 73 (42.7%)	<i>P</i> 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, <i>n</i> (%)	16 (16.3%)	8 (11%)	0.320
CVD, <i>n</i> (%)	29 (29.6%)	19 (26%)	0.608
HT, <i>n</i> (%)	70 (71.4%)	52 (71.2%)	0.978
DM, <i>n</i> (%)	34 (34.7%)	33 (45.2%)	0.164
Atrial fibrillation, <i>n</i> (%)	15 (15.3%)	10 (13.7%)	0.7691
Hypothyroidism, <i>n</i> (%)	8 (8.4%)	10 (13.7%)	0.414
Congestive heart failure, <i>n</i> (%)	11 (11.3%)	3 (4.1%)	0.090
Rheumatological diseases, <i>n</i> (%)	7 (7.1%)	8 (11%)	0.383
Malignancy history, <i>n</i> (%)	14 (14.3%)	3 (4.1%)	0.052
Chronic renal disease, <i>n</i> (%)	2 (2%)	6 (8.2%)	0.074
Pulmonary diseases, <i>n</i> (%)	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, <i>n</i> (%)	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, <i>n</i> (%)	54 (55.1%)	46 (63%)	0.299
Falls, <i>n</i> (%)	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, <i>n</i> (%)	26 (33.3%)	21 (42%)	0.321
Low gait speed, <i>n</i> (%)	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.

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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.

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Declaration of Conflicts of Interest: None.

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1.4. CoronaVac prevented Covid-19 in 93% of healthcare workers in Turkey, study shows

An article published in the journal *Human Vaccines & Immunotherapeutics* showed that CoronaVac, a vaccine from Butantan and Chinese pharmaceutical company Sinovac, was 93% effective in preventing Covid-19 in healthcare professionals in Turkey, a population with the highest level of exposure to the SARS-CoV-2 virus. The study was conducted by the School of Medicine of Yildirim Beyazit University between July and August 2021.

A total of 627 health professionals from Ankara City Hospital participated in the study, 158 men and 469 women with an average age of 35 years. Of these, 536 were immunized with CoronaVac and 91 were not vaccinated. The volunteers completed a questionnaire on demographic characteristics, vaccination status and Covid-19 history before or after immunization.

Of the individuals immunized with the Butantan vaccine, only 38 (7%) developed Covid-19 after vaccination, without the need for hospital admission, and 146 (27%) had the disease before taking the immunizing agent. Among unvaccinated

participants, about 50% (46) contracted the disease.

A phase 3 clinical trial conducted in Turkey had already demonstrated an 83.5% efficacy of CoronaVac against symptomatic cases of Covid-19 and 100% against severe disease. An effectiveness survey conducted in Chile showed that the vaccine protected 65.9% against symptomatic cases, 87.5% against hospitalizations and 90.3% against hospital admissions.

The Turkish scientists concluded that, as observed in other studies, their results prove the efficacy and safety of the immunizing agent. “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,” they say.

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Effectiveness of CoronaVac in preventing COVID-19 in healthcare workers

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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.

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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.

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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

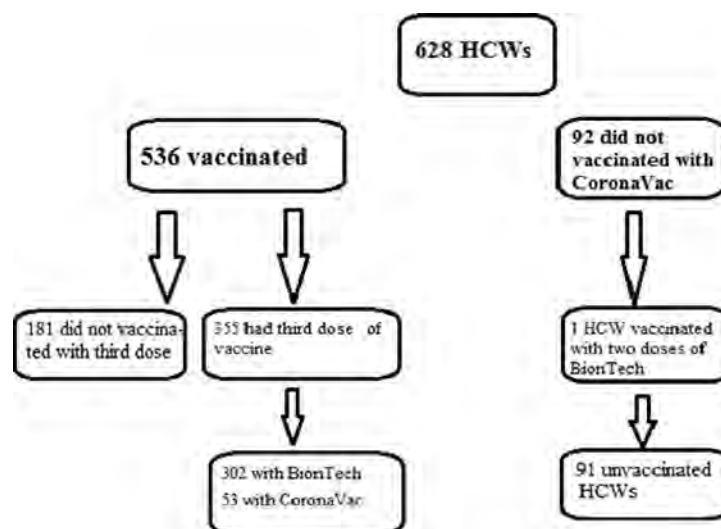


Figure 1. The vaccination status of health care workers.

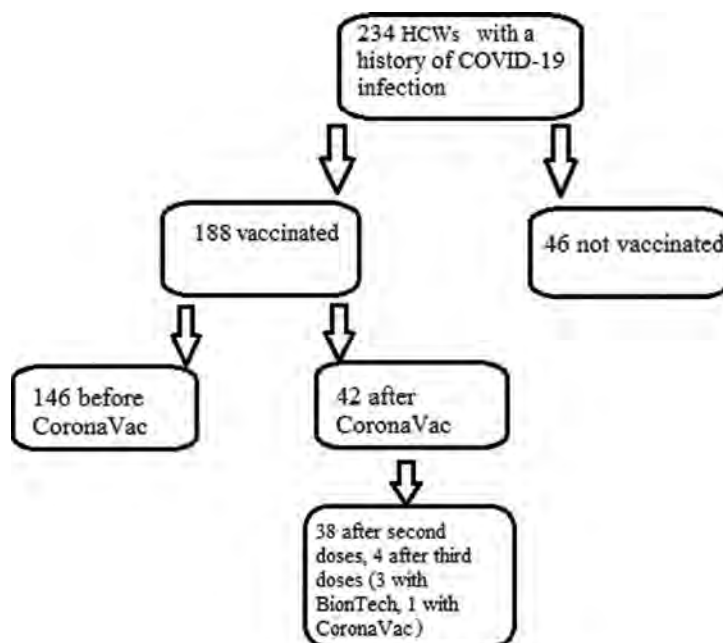


Figure 2. Vaccination history of HCWs who had experienced the COVID-19 infection.

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	<i>p</i>
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 COVID-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

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CoronaVac

What science proves

1.5. CoronaVac effective in preventing 80% of Covid-19 deaths in Indonesia

A research conducted in Bali, Indonesia, showed that CoronaVac, an inactivated virus vaccine from Butantan and Chinese pharmaceutical company Sinovac, was able to prevent more than 80% of Covid-19-related deaths. The work was published on the MedRxiv preprint platform and is the first study on the effectiveness of the immunizing agent carried out in the country – that is, with real-world efficacy data.

Scientists analyzed vaccination and diagnostic laboratory data and included 2,759 people who tested positive for Covid-19 between January and June 2021, and 2,759 controls (who did not have the disease), all of similar ages and tested within the same week. Of the positive cases, 40% had comorbidities and most were not vaccinated (53.8%).

The results showed that CoronaVac was 66.7% effective against SARS-CoV-2 infections, 71% against hospitalizations and 87.4% against death. In the case of

people over 50 years old, death prevention was even higher, reaching 90.6%.

CoronaVac was the first vaccine used in Indonesia's Covid-19 immunization program in January 2021. A phase 3 clinical study conducted in the country had already shown an efficacy of 65.3% against the disease and seroconversion (antibody production) in 87% of vaccinees, which supported the approval of its emergency use.

Studies conducted in other countries, such as Chile, Turkey and China, have shown positive results for CoronaVac. In Brazil, Project S, an effectiveness study carried out by Butantan in the municipality of Serrana, in São Paulo, showed that the vaccine prevented 80.5% of symptomatic Covid-19 cases and 95% of hospitalizations and death.

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

3

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NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.

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

66 Introduction

67 Indonesia rolled-out the first phase of mass vaccination program on January 13th, 2021, using
68 the inactivated SARS-CoV-2 CoronaVac vaccine (Sinovac Life Sciences, Beijing, China). In
69 the first phase, mass COVID-19 vaccination was targeting healthcare workers and general
70 population including adults and elderly. A phase 1/2 clinical trial in China indicated that
71 CoronaVac was tolerable, with acceptable safety and immunogenicity, and therefore supported
72 the conduct of a phase 3 clinical trial in three countries including Indonesia.¹ The efficacy of
73 CoronaVac varied among the countries, ranging from 50.7% to 84% against SARS-CoV-2
74 infection.²⁻⁴ In Indonesia, two doses of CoronaVac vaccine had an efficacy of 65.3% against
75 COVID-19 illness after 14 days of injection; this supported the approval of emergency use
76 authorization (EUA) by the Indonesia National Agency of Drug and Food Control (NADFC).
77 ^{2,5}

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

86

87 Methods*88 Setting and design*

89 Bali is a one of the popular tropical islands in Indonesia, with a total land area of 5780 km²
90 and population of more than 4.3 million.⁸ Mass COVID-19 vaccination using inactivated
91 SARS-CoV-2 (CoronaVac) was initiated by the Ministry of Health of Indonesia on January
92 13, 2021, across Indonesia, including in Bali. As of July 31, 2021, COVID-19 vaccines have
93 been given to 2.8 million (41.9%) of the targeted Balinese population. About 2.2 million
94 people aged ≥ 18 years had received at least one dose of COVID-19 vaccines (Supplementary
95 Figure S1); 1.2 million (43.1%) had received at least one dose of inactivated CoronaVac
96 vaccine.⁹ The observation was conducted during January 13 to June 30, 2021. During the
97 study period, the incidence of COVID-19 was increased during January-February and

98 followed with a declining trend from March to June 2021. There was a sharp increase in
99 incidence in July due to B.1.617.2 (Delta) variants (Supplementary Figure S2, Figure S3).
100 We conducted a record-based retrospective, test-negative, matched case control (1:1) study
101 to evaluate the effectiveness of inactivated CoronaVac vaccine in preventing laboratory
102 confirmed SARS-CoV-2 infection, among adults (aged ≥ 18 years) during January to June
103 2021. This test negative design was chosen due to several reasons. First, accessibility to
104 individual data who were laboratory-tested for SARS-CoV-2 was feasible through the
105 national surveillance system; second, it was cost-effective and allowed for faster results; and
106 lastly, the design could better control for potential biases, including healthcare seeking
107 behavior and access to COVID-19 testing.¹⁰

108

109 *Data sources*

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

118

119 *Selection of cases and matched controls*

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

131

132 *Vaccination status*

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

139

140 *Statistical analysis*

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

154

155 **Results**

156 *Study population*

157 Total 109,050 RT-PCR test results were retrieved during the January 13 to June 30, 2021
158 (Figure 1). Of which, 14,168 subjects were eligible for inclusion in the study. Of these, 2886
159 (20.4%) were having positive RT-PCR test for SARS-CoV-2 and 11,282 (79.6%) were tested
160 negative for SARS-CoV-2 infection. From this, total 5518 (39%) subjects were defined as case-
161 control pairs after matching.

162 Characteristics of the study population and matched case-control were presented in Table 1.
163 The median age of the overall study population (n=14,168) was 50 years (interquartile range,
164 31-65 years); 50.8% were aged ≥ 50 years. Thirty percent (n=4259) resided in Denpasar city.

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

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

173

174 *Vaccine effectiveness*

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

182 The adjusted VE in preventing COVID-19-related hospitalization and death was 71.1%
183 (95%CI: 62.9%-77.6%) and 87.4% (95%CI: 65.1%-95.4%), respectively, at 14 days after the
184 second dose (Table 3). Partial vaccination was less protective against COVID-19-related
185 hospitalization and death among people aged 18 and older (Supplementary Table S2). Vaccine
186 effectiveness against hospitalization was higher 79.4% (95%CI: 70-85.9%) in group of people
187 aged ≥ 50 years relative to people aged 18-49 years (65.8%; 95%CI:49.1-77%). Death due to
188 COVID-19 was not significantly associated with full vaccination in people aged 18-49 years
189 (VE:65.7%, 95%CI: -99.5% to 94.1%). This result could be happened due to an effect of small
190 sample size and small number of events (mortality). Among older population, vaccine
191 effectiveness was 90.6% (95%CI:61.5-97.7%) in preventing death due to COVID-19.

192 To address potential issues related to the small sample size and rare event or outcome, we
193 conducted an additional analysis using study population (n=14,168) (Supplementary Table S3,
194 Table S4). Overall, the vaccine effectiveness was 65.4% (95%CI: 59.5-70.4%) in preventing
195 COVID-19 infection; 78.1% (95%CI: 73.6-81.9%) in preventing hospitalization; and 93.9%
196 (95%CI: 85-97.6%) in preventing COVID-19-related death in adults aged 18 years or older at
197 14-days after second dose. The effectiveness against both hospitalization and death due to

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

201

202 Discussion

203 High proportion of inactivated vaccine of CoronaVac have been used in national COVID-19
204 vaccination program in Indonesia. So far, this is the first health record-based observational
205 study on assessing 'real-world' COVID-19 vaccine performance against laboratory-confirmed
206 SARS-CoV-2 symptomatic infection, hospitalization, and death in Indonesian population. The
207 study showed that complete regimen of CoronaVac vaccine appeared to be protective against
208 COVID-19 infection about 66.7% (58.1-73.5%) among Balinese adults aged ≥ 18 years, which
209 is consistent with the results of phase III trial.⁵ Yet, the effectiveness was reduced by age.
210 Furthermore, vaccination with CoronaVac vaccine was highly effective in preventing COVID-
211 19-associated hospitalizations and death. This demonstrates that CoronaVac vaccination could
212 reduce the risk of development severe COVID-19, especially in older population.

213 Our 'real world' estimated VE against symptomatic COVID-19 infection was consistent with
214 that randomized controlled trials of vaccine efficacy in Indonesia (65.3%; 95% CI: 20%-85.1%).
215 ² Yet, it was lower than that of vaccine efficacy reported in Turkey (83.5%; 95% CI: 65.4%-
216 92.1%)³ and higher than that efficacy reported from Brazil (51%; 95% CI: 36%-62%) at 14 days
217 after the second vaccination.¹³ Chilean cohort VE study involving general population aged ≥ 16
218 years old also demonstrated the VE was 65.9% (95% CI: 65.2%-66.6%) against COVID-19
219 infection. While a TND case control study in Brazil demonstrated that two dose regimens of
220 CoronaVac was effective in reducing risk of symptomatic COVID-19 (46.8%; 95% CI: 38.7%
221 to 53.8%) at ≥ 14 days after the second dose among older population aged ≥ 70 years.⁷

222 Our study showed that inactivated CoronaVac vaccine was also effective in preventing
223 hospitalization and COVID-19-associated death. We estimated that CoronaVac vaccine was
224 71.1% for the prevention of hospitalization and 87.4% for the prevention of COVID-19-related
225 death. None has reported CoronaVac VE estimates against these two outcomes in Indonesia.
226 Our VE estimates are dissimilar to estimates that have been reported elsewhere. A cohort study
227 in Chile among adults aged ≥ 16 years showed that CoronaVac vaccine was 87.5% (95% CI,
228 86.7 to 88.2) effective against hospitalization and 86.3% (95% CI, 84.5 to 87.9) effective
229 against COVID-19-related death.^{6,14} While a TND study in Brazil among elderly aged 70 years
230 or older, CoronaVac was effective against COVID-19-related hospitalization (55.5%; 95% CI:

231 46.5% to 62.9%) and death (61.2%, 95%CI: 48.9% to 70.5%) at ≥ 14 days after the second dose,
232 respectively.⁷ This discrepancy might be due to several factors including variation in study
233 population and design (e.g., targeted age group, sample size) and difference in risk of
234 transmission or epidemiological setting (e.g., a TND study in Brazil was conducted when P.1
235 or Gamma variant was predominant).

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

254

255

256 **Conclusions**

257 The two-dose regimens of inactivated CoronaVac vaccine was effective in preventing COVID-
258 19 infection, hospitalizations for COVID-19 and COVID-19-related death in adults aged 18
259 years or older. Full vaccination was significantly reduced the risk of severe outcomes especially
260 among 50 years or older.

261

262 **Author contributions**

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

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

269

270 **Ethic statement**

271 The protocol of the study was approved by the Health Research Ethics Committee, National
272 Institute of Health Research and Development, Ministry of Health, Indonesia (LB.
273 02.01/2/KE.533/2021). The study was considered exempt from informed consent. No human
274 health risks were identified.

275

276 **Conflict of interest**

277 The authors declare that they have no competing interests. Anton Suryatma, Raras Anasi, Miko
278 Hananto, Asep Hermawan, Ririn Ramadhany, Irene Lorinda Indalao, Agustiningsih
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284 have any share or financial interests in Sinovac Life Sciences or parent companies.

285

286 **Data availability statement**

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

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307

308

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 321 Placebo-Controlled Phase III Clinical Trial to Evaluate the Efficacy and Safety of
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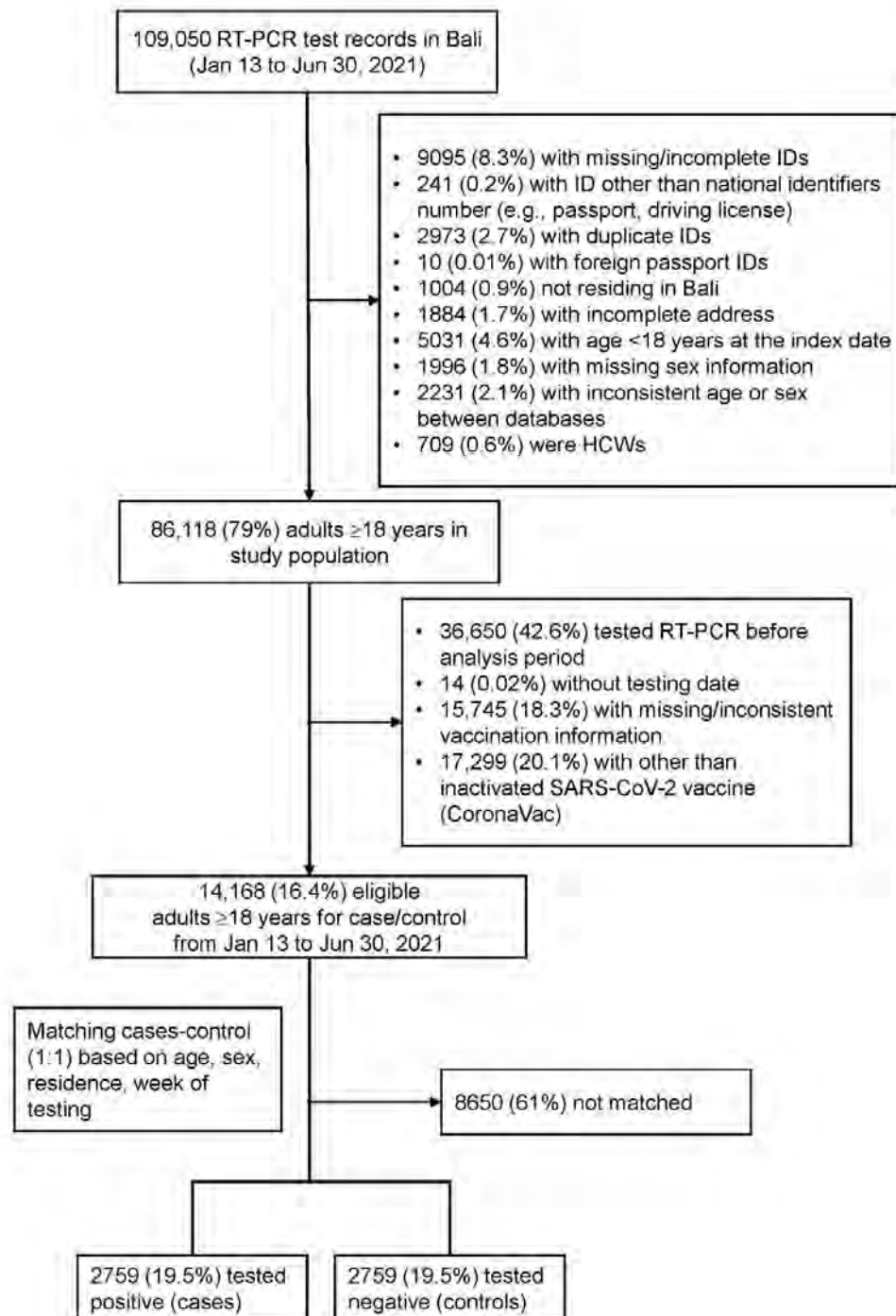


Figure 1. Flowchart for case and control selection

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

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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)

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417 **Table 2.** CoronaVac vaccine effectiveness against laboratory-confirmed SARS-CoV-2
 418 infection in adults aged ≥ 18 years in Bali, Indonesia
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Vaccination status	Cases	Controls	Vaccine effectiveness (95% CI)	
			Unadjusted	Adjusted*
Unvaccinated	1771 (64.2)	1469 (53.3)	Ref	Ref
0-13 days after 1 st dose	216 (7.8)	193 (7)	22.9 (2.4- 39)	14.5 (-11 – 34.2)
≥ 14 days after 1 st dose	319 (11.6)	272 (9.9)	24.1 (7.4-37.8)	10.5 (-12 – 28.6)
0-13 days after 2 nd dose	74 (2.7)	158 (5.7)	67 (55.3-75.6)	63.1 (48.5-73.6)
≥ 14 days after 2 nd dose	379 (13.7)	667 (24.2)	73.4 (78.4-67.2)	66.7 (58.1-73.5)

420 *Included covariates: age (as continuous), presence of comorbidities and prior SARS-CoV-2
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460 **Table 3.** CoronaVac vaccine effectiveness in preventing hospitalization and COVID-19-
 461 related death in adults aged ≥ 18 years in Bali, Indonesia
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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 1 st 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 1 st 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 2 nd 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 2 nd dose	166 (6.7)	880 (29)	71.1 (62.9-77.6)	4 (0.8)	1042 (20.7)	87.4 (65.1-95.4)

463 Abbreviations: VE, vaccine effectiveness; CI, confidence interval

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

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499 **Supplementary file**

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501 **Table S1.** CoronaVac vaccine effectiveness against laboratory-confirmed SARS-CoV-2

502 infection in adults (aged ≥18 years), by age group, in Bali, Indonesia

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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 1 st dose	133 (15.7)	122 (14.4)	57.2 (37.6-70.6)	48.3 (22.6-65.5)
≥14 days after 1 st dose	144 (17)	131 (15.5)	65.7 (49.4-76.8)	55.1 (32-70.4)
0-13 days after 2 nd dose	39 (4.6)	79 (9.3)	80.3 (68-87.9)	78.2 (63.6-87)
≥14 days after 2 nd 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 1 st dose	83 (4.3)	71 (3.7)	-5 (-46.6-24.8)	-10.6 (-64.3-25.5)
≥14 days after 1 st dose	175 (9.2)	141 (7.4)	-2 (-30 -20)	-13.2 (-50.6 -14.9)
0-13 days after 2 nd dose	35 (1.8)	79 (4.1)	61.7 (42.3-74.6)	58.1 (32.4-74)
≥14 days after 2 nd dose	95 (4.9)	247 (12.9)	74.3 (65.2-80.9)	69.9 (57.7-78.7)

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

525 **Table S2.** CoronaVac vaccine effectiveness in preventing hospitalization and death due to
 526 COVID-19 in adults (aged ≥ 18 years), by age group, in Bali, Indonesia (n=5518)
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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 1 st dose	53	202	34.5 (-1-57.5)	0	255	NA
≥ 14 days after 1 st dose	65	210	32.9 (-1.9-55.8)	1	274	78.7 (-84.6-97.6)
0-13 days after 2 nd dose	16	102	64.9 (32.7-81.7)	1	117	51.3 (-330-94.5)
≥ 14 days after 2 nd 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 1 st dose	71	83	14.1 (-28.1-42.4)	10	144	35.7 (-26.1-67.3)
≥ 14 days after 1 st dose	153	163	34.7 (11.2-51.9)	14	302	57.2 (24.7-75.7)
0-13 days after 2 nd dose	34	80	55.1 (26.5-72.6)	2	112	78.9 (12.7-94.9)
≥ 14 days after 2 nd dose	67	275	79.4 (70-85.9)	2	340	90.6 (61.5-97.7)

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552 **Table S3.** Assessment of CoronaVac vaccine effectiveness against laboratory-confirmed
 553 SARS-CoV-2 infection, hospitalization and death in adults aged ≥ 18 years in Bali, Indonesia,
 554 based on study population (n = 14,168)
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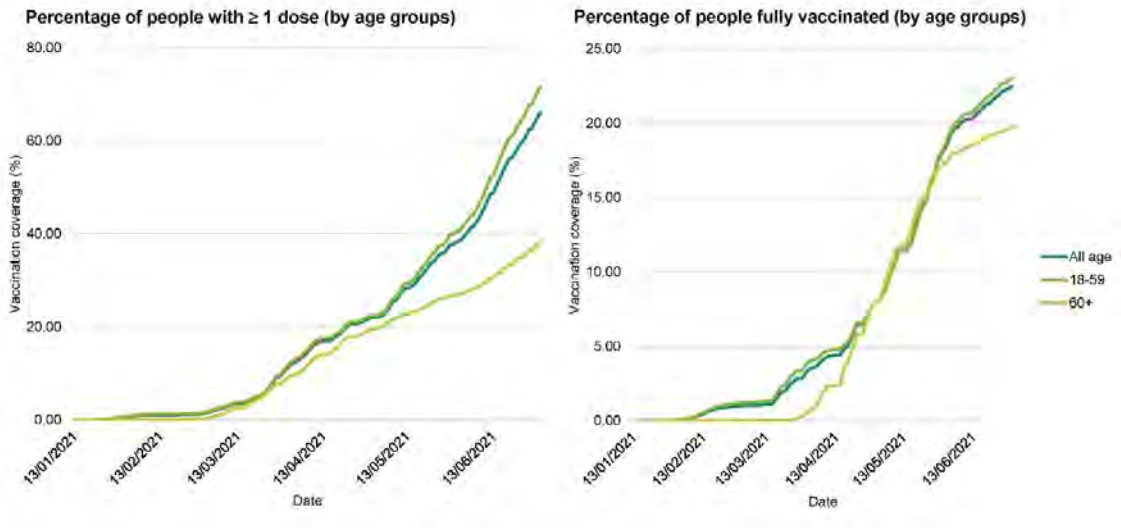
Vaccination status	COVID-19 (n/N)	VE* % (95%CI)	Hospitalization (n/N)	VE* % (95%CI)	COVID-19-related death (n/N)	VE* % (95%CI)
Unvaccinated	1867/6016	Ref	2841/6016	Ref	574/6016	Ref
0-13 days after 1 st dose	222/1001	6.7 (-12.1-22.3)	158/1001	27.9 (8.7-43.1)	10/1001	64.5 (31.6-81.5)
≥ 14 days after 1 st 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 2 nd 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 2 nd dose	380/4863	65.4 (59.5-70.4)	308/4863	78.1 (73.6-81.9)	5/4863	93.9 (85-97.6)

556 *Based on adjusted multivariable logistic regression, accounting for age (continuous), sex, district of
 557 residence, week of testing, presence of comorbidities, and prior infection.
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590 **Table S4.** CoronaVac vaccine effectiveness in preventing hospitalization and death due to
 591 COVID-19 in adults (aged ≥18 years), by age group, in Bali, Indonesia based on the study
 592 population (n = 14,168)
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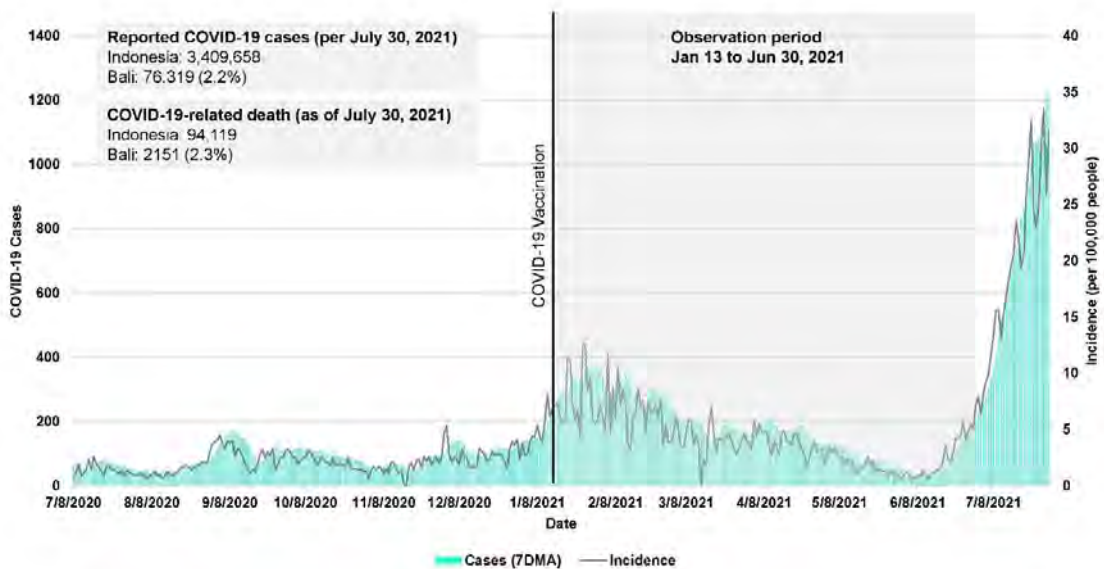
Vaccination status	Hospitalization		VE* % (95%CI)	Death		VE* % (95%CI)
	Yes (n=3718)	No (n=10,450)		Yes (n=482)	No (n=5036)	
Age group: 18-49 years (n=1694)						
Unvaccinated	187	689	Ref	14	862	Ref
0-13 days after 1 st dose	73	691	36 (8.2-55.4)	0	764	NA
≥14 days after 1 st dose	109	749	35.8 (9.3-54.5)	1	857	88 (1.6-98.5)
0-13 days after 2 nd dose	26	659	75.3 (59.4-85)	1	684	73.6 (-117.7-96.8)
≥14 days after 2 nd 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 1 st dose	85	152	12.9 (-22.6-38.1)	10	227	44.4 (-7.7-71.2)
≥14 days after 1 st dose	227	323	26.6 (6.4-42.5)	18	532	57.8 (30.9-74.3)
0-13 days after 2 nd dose	49	146	48 (21.8-65.4)	2	193	83.9 (34.1-96.1)
≥14 days after 2 nd dose	130	947	83.1 (77.9-87.1)	3	1077	94.1 (81.4-98.1)

594 *Based on adjusted multivariable logistic regression, accounting for age (continuous), sex, district of
 595 residence, week of testing, presence of comorbidities, and prior infection.
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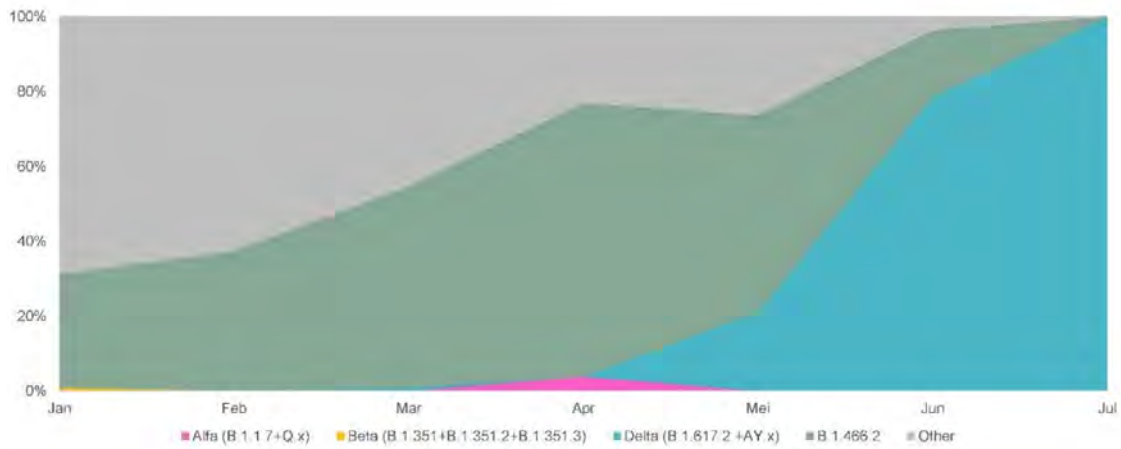
Figure S1. COVID-19 vaccination coverage in adults ≥ 18 years (any type of vaccines) during January 13 to June 30, 2021, in Bali, Indonesia (Data source: Ministry of Health of Indonesia).



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

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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).

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

66 Introduction

67 Indonesia rolled-out the first phase of mass vaccination program on January 13th, 2021, using
68 the inactivated SARS-CoV-2 CoronaVac vaccine (Sinovac Life Sciences, Beijing, China). In
69 the first phase, mass COVID-19 vaccination was targeting healthcare workers and general
70 population including adults and elderly. A phase 1/2 clinical trial in China indicated that
71 CoronaVac was tolerable, with acceptable safety and immunogenicity, and therefore supported
72 the conduct of a phase 3 clinical trial in three countries including Indonesia.¹ The efficacy of
73 CoronaVac varied among the countries, ranging from 50.7% to 84% against SARS-CoV-2
74 infection.²⁻⁴ In Indonesia, two doses of CoronaVac vaccine had an efficacy of 65.3% against
75 COVID-19 illness after 14 days of injection; this supported the approval of emergency use
76 authorization (EUA) by the Indonesia National Agency of Drug and Food Control (NADFC).
77 ^{2,5}

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

86

87 Methods**88 *Setting and design***

89 Bali is a one of the popular tropical islands in Indonesia, with a total land area of 5780 km²
90 and population of more than 4.3 million.⁸ Mass COVID-19 vaccination using inactivated
91 SARS-CoV-2 (CoronaVac) was initiated by the Ministry of Health of Indonesia on January
92 13, 2021, across Indonesia, including in Bali. As of July 31, 2021, COVID-19 vaccines have
93 been given to 2.8 million (41.9%) of the targeted Balinese population. About 2.2 million
94 people aged ≥ 18 years had received at least one dose of COVID-19 vaccines (Supplementary
95 Figure S1); 1.2 million (43.1%) had received at least one dose of inactivated CoronaVac
96 vaccine.⁹ The observation was conducted during January 13 to June 30, 2021. During the
97 study period, the incidence of COVID-19 was increased during January-February and

1.6. Study shows 99.9% effectiveness of CoronaVac in Colombian Amazon

A study published in the *Tropical Diseases, Travel Medicine and Vaccines* journal found that CoronaVac, a vaccine produced by Butantan and the Chinese pharmaceutical company Sinovac, was 99.9% effective in preventing severe cases of Covid-19 in a population in the Amazon region of Colombia, and offered protection of 94.3% against mild cases of the disease.

The descriptive observational study was conducted between February and August 2021 in the municipality of Mitú, Vaupés, with 7,849 individuals over the age of 18 immunized with CoronaVac - equivalent to 99% of the population. The region was prioritized in the vaccination campaign due to its proximity to the Brazilian state of Amazonas, where the gamma variant (P.1) of SARS-CoV-2 has emerged.

Analyses showed that after immunization, 5.7% of those vaccinated had Covid-19 and only 0.1% required hospitalization.

Among those infected under the age of 60 (406), 405 developed mild symptoms and only one had moderate symptoms. In the elderly (41), 40 had mild infection and one had severe disease.

Decrease of cases and deaths

In May 2021, there was a new peak of 200 cases of Covid-19 in Mitú. "This increase was much lower than the August 2020

peak, when 327 cases were reported," the researchers point out in the paper. The mortality rate was also reduced from 2.2 percent to 0.22 percent in the comparison between the two periods.

In addition, when the peak of immunized individuals was reached, there was a reduction of 72% in Covid-19 cases in the municipality.

Scientists highlight that the cases in the vaccinated population of Mitú can be attributed to the high circulation of the gamma variant at the time. However, the study shows that CoronaVac was able to control the severity of cases and mortality related to this strain.

Butantan and Sinovac vaccine represents 40% of the Covid-19 vaccines used in Colombia and has already had more than 1.8 billion doses applied worldwide. In Brazil, 100 million doses were applied.

"This vaccine platform consists of inactivated SARS-CoV-2 virus and has already had proven safety, effectiveness and immunogenicity. The strategy has also been used successfully in Serrana, Brazil," reports the article, referring to the results of Project S, a clinical effectiveness study conducted by Butantan in a municipality in São Paulo's countryside.

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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 Miller³, Camilo Guzmán¹, Ricardo Rivero¹, Bertha Gastelbondo¹, Jorge Miranda¹, Ketty Galeano¹, Jhon Montaña-Restrepo³ 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

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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 Mitú. This figure showed that Mitú 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 Mitú, 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)	
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	
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.

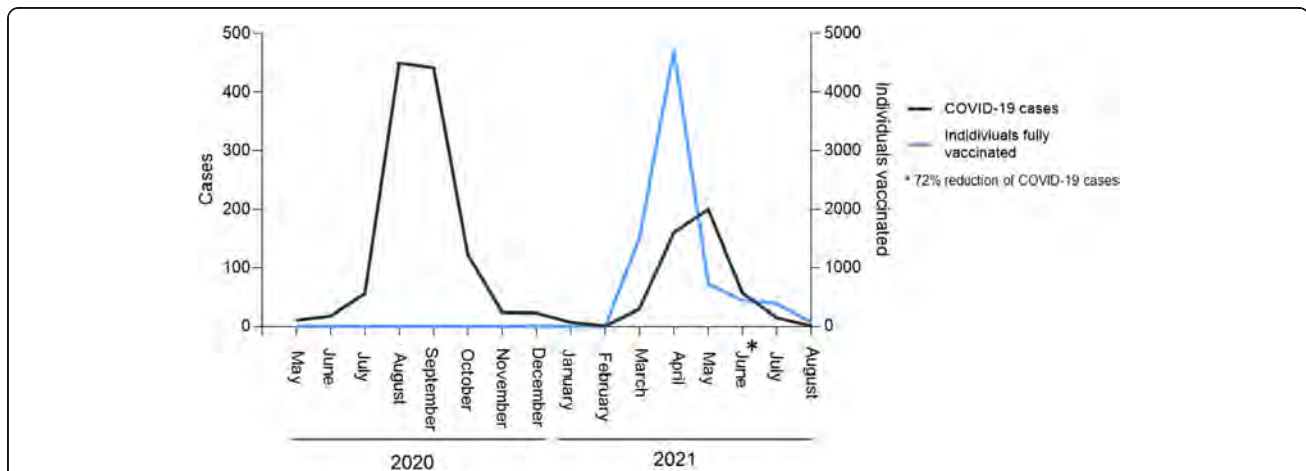


Fig. 2 Characterization of COVID-19 cases in Mitu municipality. This figure showed a first peak or wave of cases of COVID-19 in August 2020, with a significant drop of cases in November 2020. The vaccination in this municipality started in February 2021 and a second wave was observed between April–May 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 Mitu 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.

Abbreviations

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

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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.

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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

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>)

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.

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CoronaVac

What science proves

1.7. Study in Serrana shows effectiveness of 80,5% from CoronaVac against cases of Covid-19 and 94,4% against deaths; vaccination also protected the non vaccinated against the gamma variant

The data of the first analysis from the Project S, effectiveness study of CoronaVac vaccine that Butantan conducted in the city of Serrana, located in the countryside of São Paulo, shows a direct effectiveness of 80,5% against symptomatic cases of Covid-19, 95% against hospitalizations and 94,4% against deaths. The research also indicates that, with 52% of the population vaccinated, the indirect effects began to manifest, including protecting those who weren't immunized. Besides, at the time of the study (between february and may of 2021), the majority of cases were induced by the gamma variant (P.1, amazonic) of SARS-CoV-2, which put on evidence again that CoronaVac is efficient against this strain - that predominated in Brazil during the whole first semester of 2021.

The results of the research, conducted by scientists from Instituto Butantan, from the State Hospital of Serrana, of the Medicine School from Ribeirão Preto of the University of São Paulo and of the Municipal Secretary of Health from Serrana, are described in

the article "Project S: a stepped-wedge randomized trial to assess CoronaVac effectiveness in Serrana, Brazil", disclosed on the preprint platform SSRN.

The Project S - a stepped-wedge randomized trial - is the first clinical trial that demonstrated the efficiency of a vaccine in the real world and its indirect effect on the non vaccinated population, being held during a pandemic and without using a control group. The research is pioneer in demonstrating that a vaccine of inactivated virus used as an emergency measure of primary public health can change the course of an epidemic. In addition, the study shows that vaccines are essential to contain the number of cases and the viral transmission and to control the devastating effects of Covid-19.

The volunteers in Project S were vaccinated with CoronaVac, a vaccine from Butantan and the chinese pharmaceutical Sinovac, in a scheme of two doses with a gap of four weeks. In total, completed the vaccinal scheme 81,3% of the adult population and 60,9% of the urban population from

Serrana, which equals to 27 thousands of people. Of those, 16% were elderies, older than 60 years.

The general efficacy of the vaccine was estimated by comparing the incidence of cases before and after vaccination for the whole urban population. The direct efficacy was evaluated in the relation between the incidence of cases on individuals that were fully vaccinated and non vaccinated. Among those vaccinated, the direct effectiveness was 80,5% (IC 95%, 75,1 to 84,7) in the prevention of symptomatic cases; 95% (IC 95%, 86,9 to 98,1) against hospitalization; and 94,9% (IC 95%, 76,4 to 98,9) to prevent deaths. During the period of the study, 1,447 cases of Covid-19 were reported in Serrana; from that number, 361 (24,9%) were sequenced, indicating an incidence of the gamma variant from 92% to 100% in the city.

When analyzing the impact of the vaccination in individuals older than 60 years, the direct effectiveness of CoronaVac stays very high: 86,4% (IC 95%, 74,5 to 93) in the prevention of symptomatic cases,

96,9% (IC 95%, 86,1 to 99,3) against hospitalizations and 96,9% (IC 95%, 73,9 to 99,6) to prevent deaths.

The scientists say that it's not possible to determinate a minimum level of immunization necessary to control the Covid-19 on a location, but the results of the Project S demonstrated that when 52% of the population had received the two doses of the vaccine, the indirect effects of protection began to appear on the other groups that haven't completed the immunization yet - suggesting an immunization indicator to control the gamma variant of SARS-CoV-2. Besides, during the study period, the number of infections among children was also reduced, indicating the indirect effect of CoronaVac in this population, which was not immunized. However, researchers say that the direct effects of vaccination were higher than the indirect, reinforcing the necessity of vaccinating the highest number of people as fast as possible.

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Original Research

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

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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)

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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 $100 \times (1 - IRR)$ and 95% CIs for vaccine effectiveness estimated as $100 \times (1 - \text{upper or lower bounds of 95\% CI for IRR})$, where:

$$IRR = \frac{(\text{number cases}_{intervention\ period}) / (\text{total person} - \text{days at risk}_{intervention\ period})}{(\text{number cases}_{control\ period}) / (\text{total person} - \text{days at risk}_{control\ period})},$$

and the 95% CI for IRR was calculated as, $e^{\{\log(IRR) \pm 1.96 \times SE(\log(IRR))\}}$ with the standard error for $\log(IRR)$:

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

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

$$dVE = 1 - \frac{(\text{cases}_{vaccinated}) / (\text{Total person} - \text{days at risk}_{vaccinated})}{(\text{cases}_{unvaccinated}) / (\text{Total person} - \text{days at risk}_{unvaccinated})}.$$

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

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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



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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



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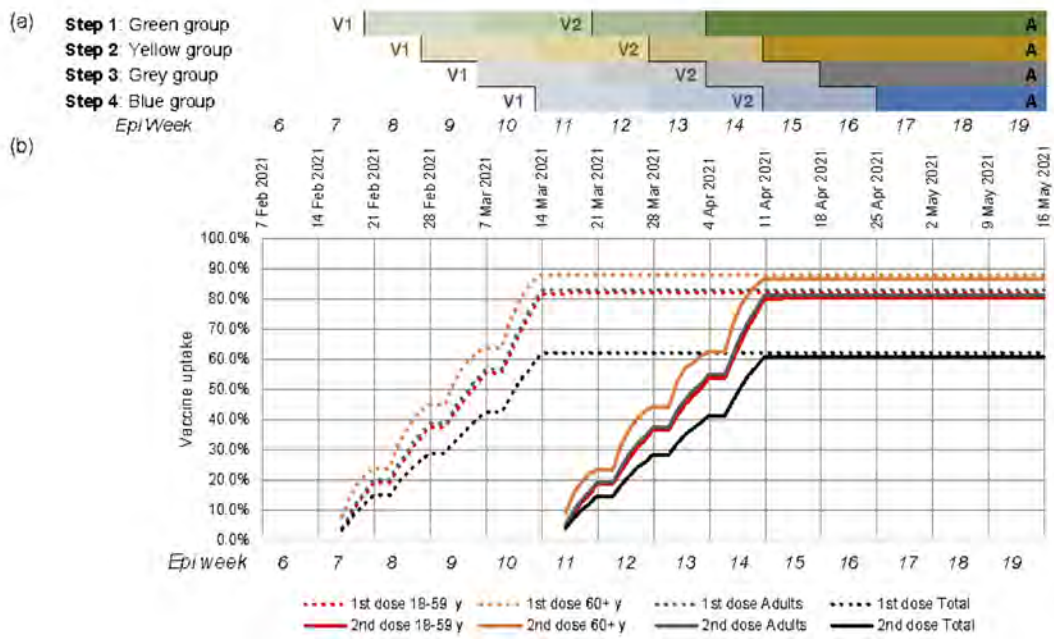
Figure legends

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.



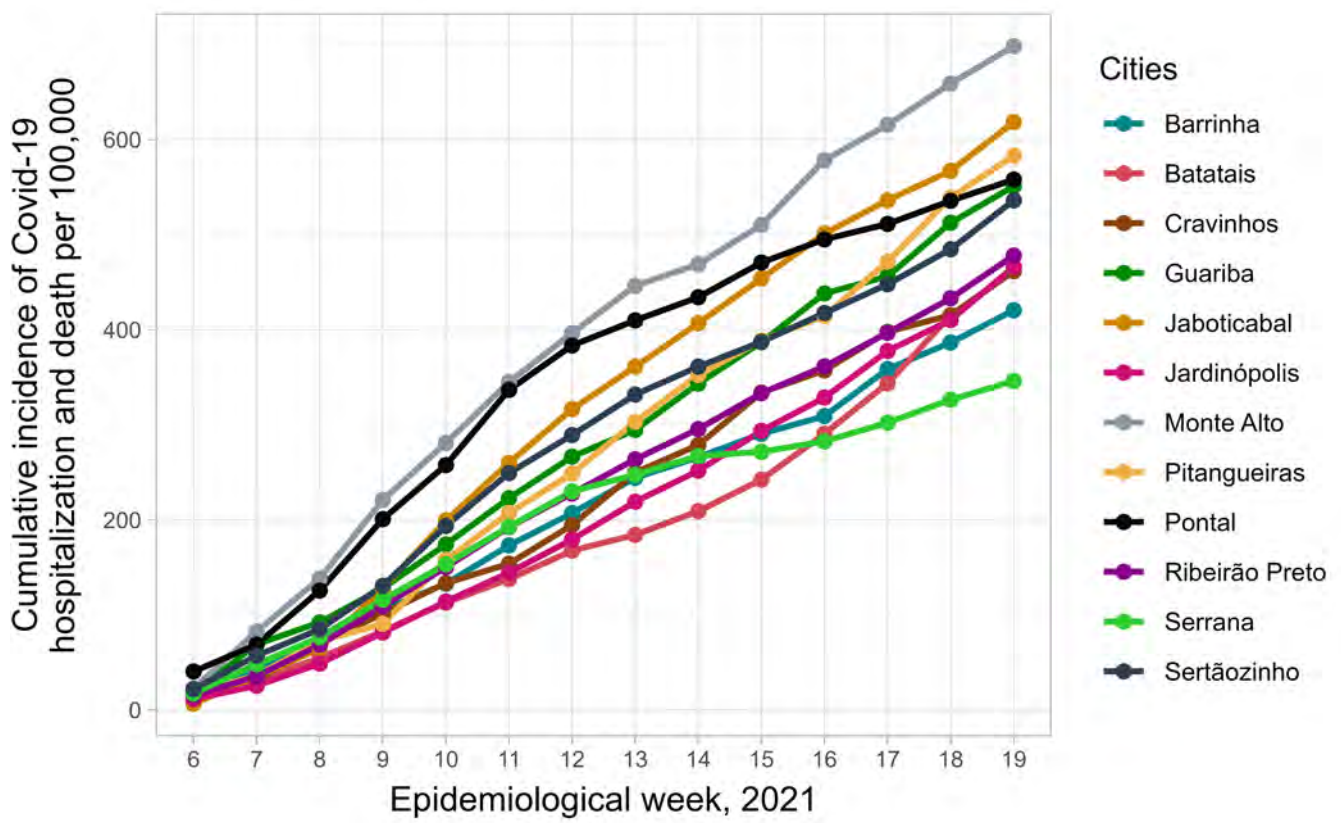


Table 1. Characteristics of the Study Population, Overall, per Group, and According to Vaccination Status, Serrana, Brazil, 2021.

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 generates high antibody responses in healthcare workers with and without prior Covid-19 infection, say studies from Turkey

Two researches conducted in Turkey showed that CoronaVac, the vaccine of Butantan and Sinovac, produces effective humoral immunity in healthcare workers with and without a history of Covid-19, with seroconversion rates above 99%. In individuals who have already had the infection, the level of antibodies produced was 1.3 times higher than in those who have never been infected.

The first study, published in July 2021, analyzed 730 healthcare workers: 103 (14%) had been previously infected with mild or asymptomatic SARS-CoV-2, and 627 (83%) had not been infected. All individuals were immunized with two doses of CoronaVac at a 28-day interval.

One month after the second dose, specific IgG antibodies to Spike protein were detected in both groups - parallel studies of phases 1 and 2 showed seroconversion in 98% of healthcare workers.

In previously infected people, antibody levels were significantly higher (1220 AU/mL) than in the second group (913 AU/mL). Furthermore, there was no difference in vaccine-related adverse reactions between previously infected and uninfected individuals, both in the first and in the second dose.

On the other hand, the second study, published in November 2021, was conducted with 330 healthcare workers of the Istanbul University Cerrahpasa,

Faculty of Medicine, aged 19 to 65, who were immunized with CoronaVac. Of these, 255 had never had the disease and 75 had a previous history of Covid-19 (five asymptomatic, 36 mild, 31 moderate and three severe cases).

Samples collected 28 days after the second dose showed seroconversion of IgG antibodies in 100% of the previously infected and 99.2% of the uninfected. In all study participants, the efficacy rate of CoronaVac was 99.4%.

In the group without prior infection, the mean antibody titer was 48.4 AU/mL after the first dose, which increased to 707.1 AU/mL after the second dose. Among those with a prior history of Covid-19, the mean antibody titer was 301.9 AU/mL before vaccination, rising to 1331.2 AU/mL after the first dose and remaining at similar levels after the second dose.

In summary, participants who have had Covid-19 developed significantly higher seroconversion rates after the first dose of the vaccine than the participants with no history of the disease, but the rates of antibody development after full immunization were similar, between 99% and 100%.

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



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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-naïve 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-naïve 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.

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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 world-

wide, 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

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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-

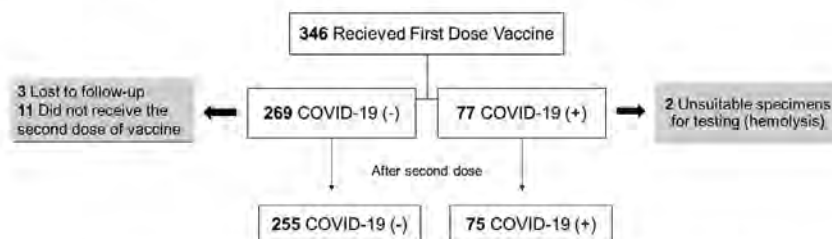


Fig. 1. Flowchart of volunteers participating in the Inactive SARS-CoV-2 Vaccine Efficacy Study.

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 39.52 ± 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			
O+	69 (32,1)	17 (25,4)	,815
O-	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 Median (IQR 25–75)	Prior History of COVID-19 Median (IQR 25–75)	p
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 / milliliter ; IQR : Inter Quantile Range.

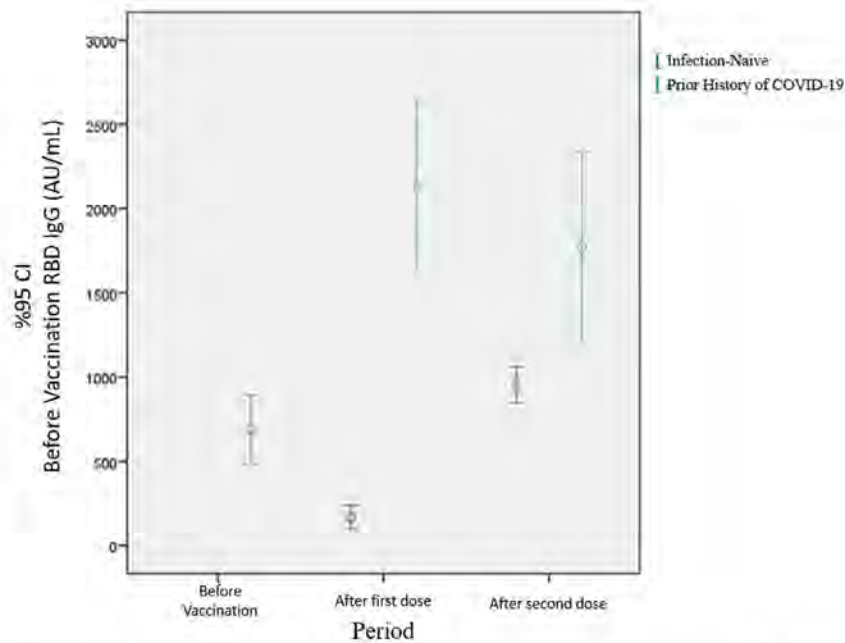


Fig. 2. SARS-CoV-2 IgG averages in blood samples taken at different times from healthcare workers who have prior history of COVID-19 and who are infection naïve.

infection-naïve group ($r = -0.15$ $p < 0.05$). When evaluated in terms of comorbid conditions; It was found that COVID-19 infection-

naïve 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)	,041	62	1056,6(495,4–1781,7)	,0203
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.

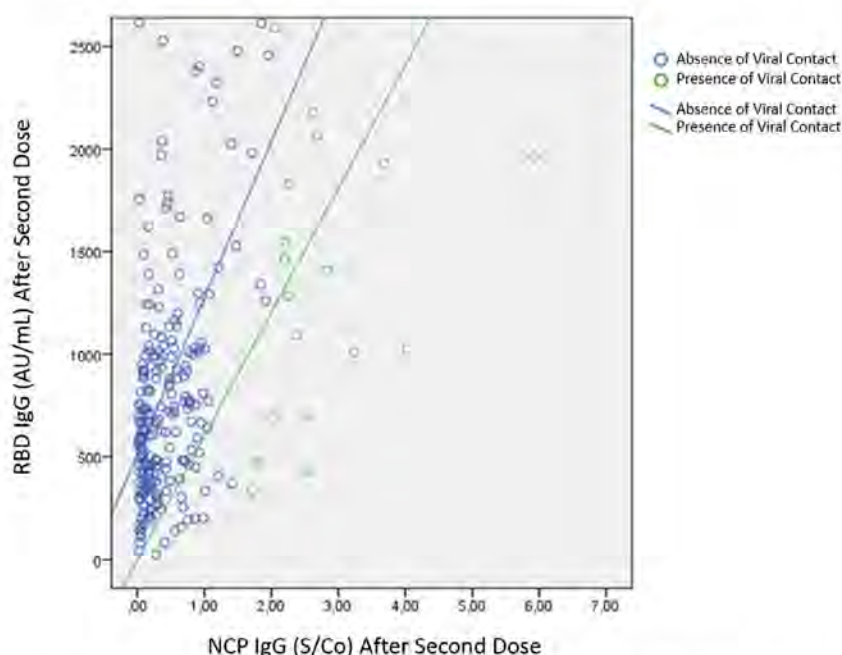


Fig. 3. SARS-CoV-II (RBD) IgG results by depending on viral contact in the Infection-Naive group.

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 pro-

grams. 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-naïve 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 asymptotically and very recently, probably before the second vaccination or more earlier 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-naïve 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.

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

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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 (10%) 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.

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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

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-naïve 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 χ² 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

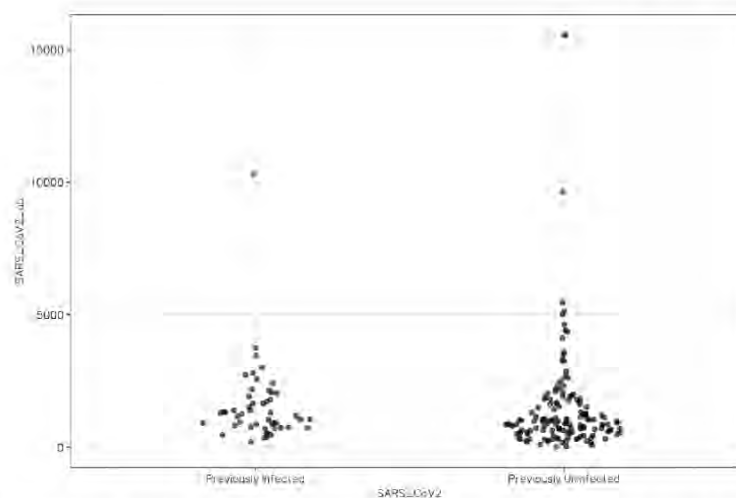


Figure 1. Anti-SARS-CoV-2 antibody responses after 2 doses of vaccine in health care workers concerning previous infection status. Anti-RBD antibody (Arbitrary unit per ml)

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-naïve 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-naïve ($n = 228$) 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-naïve individuals.¹³

In contrast, Tazuin et al. investigated humoral and T cell immune responses in cohorts of SARS-CoV-2 naïve ($n = 16$) 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-naïve 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; naïve 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 pre-existing 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.

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1.9. CoronaVac induces the production of specific antibodies against the main proteins of the SARS-CoV-2 virus

A Brazilian study published in *Diagnostic Microbiology and Infectious Disease* journal identified a high production of IgG specific antibodies against the Spike (S) protein and against nucleocapsid (N) protein of SARS-CoV-2 on healthcare workers vaccinated with CoronaVac, with and without previous infection with Covid-19, and with and without comorbidities. These are the most important proteins of the virus that induce the higher immune response.

Published in November 2021, the analysis was conducted by scientists of the Federal University of Paraná (UFPR) and from the National Center of Research in Energy and Materials of Campinas. The 133 volunteers were professionals of the Complex Clinical Hospital of UFPR, from Curitiba, aged between 25 to 59 years, being nine immunosuppressed and 124 without comorbidities. The individuals were also divided into another two groups: those that presented positive serology for Covid-19 before the vaccination (16) and those that never had the disease (117).

A robust production of specific IgG antibodies for the S protein, that allows the virus to enter the human

cells, was detected in 97% of the participants two weeks after the second dose. Besides, 52% presented IgG antibodies against the N protein - that's because CoronaVac is an inactivated vaccine that contains the whole virus, therefore capable of promoting a wider immune response, not restricted to only one protein.

The levels of antibodies produced were similar, independent if the participant has had Covid-19 before or not. On immunosuppressed individuals, in general, the immune response was also similar to the group without comorbidities.

The researchers call the attention to the rates of seroconversion observed for the protein N, the most conserved and stable of the virus. "Since this protein presents a low level of mutation, specific antibodies for that protein can be viable to combat the variants that have a high level of mutation in the S protein", they said. However, they reinforce that more studies must be done to understand the protecting effect of specific antibodies against the other proteins of the virus.

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Original Article

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



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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.

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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

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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 ($P = 0.3704$).

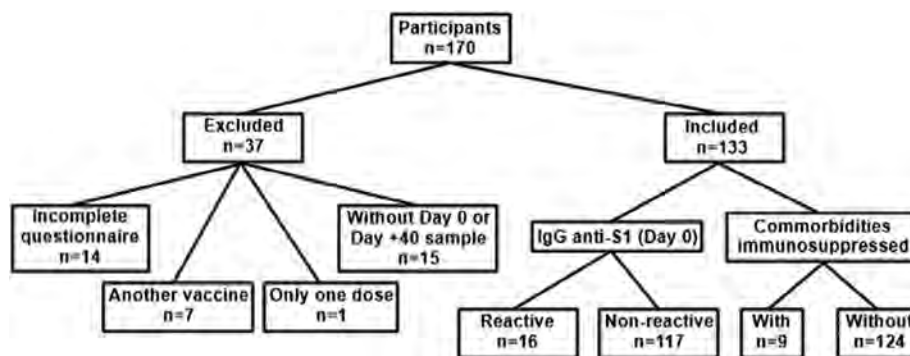


Fig. 1. Participants included and excluded in the study and division of groups for analysis. Comorbidities (immunosuppressive) included: Immunosuppressive drugs use, Crohn's disease, bariatric surgery, HIV and Diabetes.

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

	IgG Anti-S1 (Day 0)		P value	Comorbidities immunosuppressive ^b		P value
	Reactive n (%)	Nonreactive n (%)		With n (%)	Without n (%)	
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/ μ L).

^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

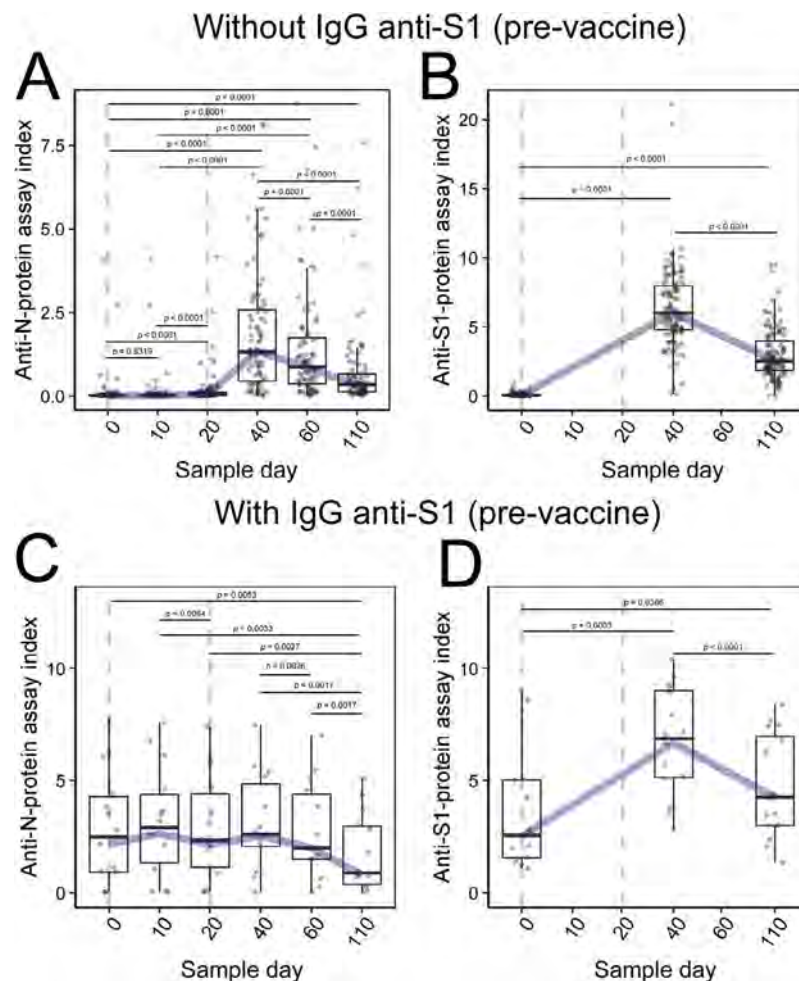


Fig. 2. Antibody rates in the S1-protein IgG seroconverted/not seroconverted groups at day 0. Boxplot graph presents median (line dividing the box), interquartile range (box), maximum value (line above the box), and minimum value (line below the box). The line connecting the boxes represents the trend 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.

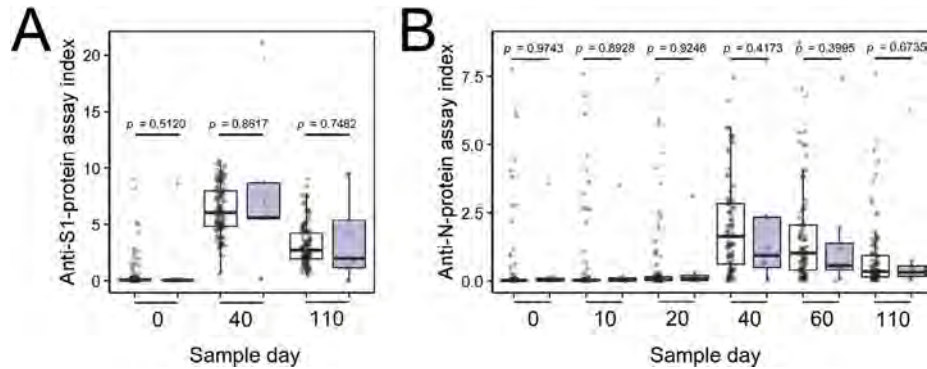


Fig. 3. Antibody rates for participants with and without immunosuppression. White boxes indicate nonimmunosuppressed participants. Gray boxes indicate immunosuppressed participants. (A) S1-protein IgG evaluation. (B) N-protein IgG evaluation.

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

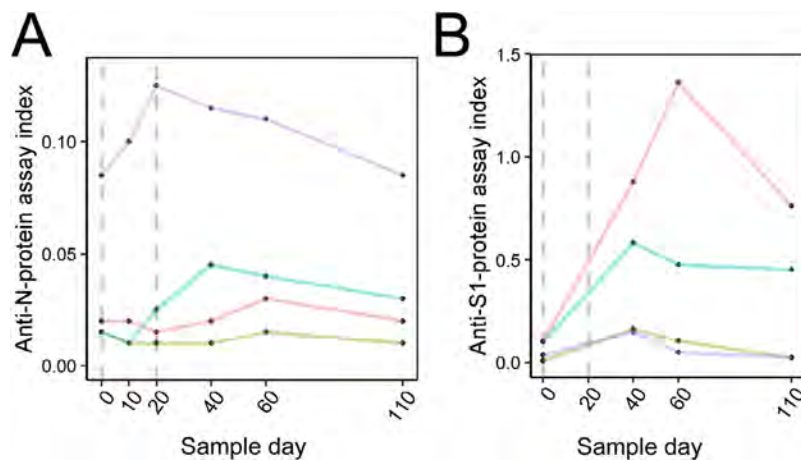


Fig. 4. Antibody rates for participants without seroconversion on day +40. Purple and green lines represent the participants with Rituximab treatment. The dotted line represents the days of the vaccine application (2 doses). (A) N-protein IgG evaluation. (B) S1-protein IgG evaluation (color version of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

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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.

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1.10. CoronaVac induces immunological memory that is efficient and similar to the one from convalescent patients, shows chinese study

An article published in the Clinical Microbiology and Infection journal showed that CoronaVac, the vaccine from Butantan and Sinovac, presents a high efficacy in the humoral response (antibodies production) and in the cellular response (T cells CD4+ and CD8+) against the SARS-CoV-2, and promotes immunological memory comparable to the convalescent patients. The study was conducted by Chinese researchers from Nanjing University between January and February of 2021.

The scientists analyzed the immune response of 100 healthcare workers (37 men and 63 women) aged between 23 and 59 that were vaccinated with CoronaVac. Blood samples were collected before the first dose (T1), two weeks after the first dose (T2), two weeks after the second dose (T3) and 8 to 10 weeks after the second dose (T4).

All participants presented seroconversion (antibodies production) 14 days after the second dose - 98% of the individuals produced specific IgG antibodies against the Spike protein and 85% had antibodies capable of neutralizing SARS-CoV-2.

In addition, scientists detected powerful responses of the memory T cells CD4+ and CD8+, with comparable levels to those found in recovered patients that already had Covid-19. According to the authors, specific T cells CD4+ and CD8+ have already been associated with the reduction of the severity of the disease.

Volunteers also had memory B cells (that produce antibodies) that remained until the final analysis, eight to ten weeks after the second dose. Those cells are responsible for recognizing the virus antigens and

are capable of quickly reacting to the infection.

The researchers say that this study brings new information about the immunobiology of inactivated vaccines and may have implications for vaccine strategies in the future. “We identified memory T cells CD4+ associated with memory B cells specific for Spike protein and with memory T cells CD8+, indicating a convergent development of the adaptive humoral and cellular immunity”.

Factors that interfere in the immune response

Half of the participants received the second dose of the vaccine with a 14-21 days interval, while the other 50 received the second dose from 22 to 30 days after the first dose. The group immunized with a longer interval between doses had a higher

level of neutralizing antibodies and a higher percentage of specific B cells for the Spike protein and of memory T cells CD4+ and CD8+.

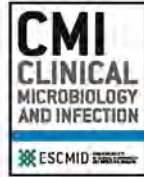
The age also influenced the immune response: people between 20 and 40 years old presented higher mean titers of neutralizing antibodies (GMT 42) than individuals older than 40 (GMT 26). Despite that, both groups had similar levels of specific IgG antibodies for the Spike protein.

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Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine

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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**

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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

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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,

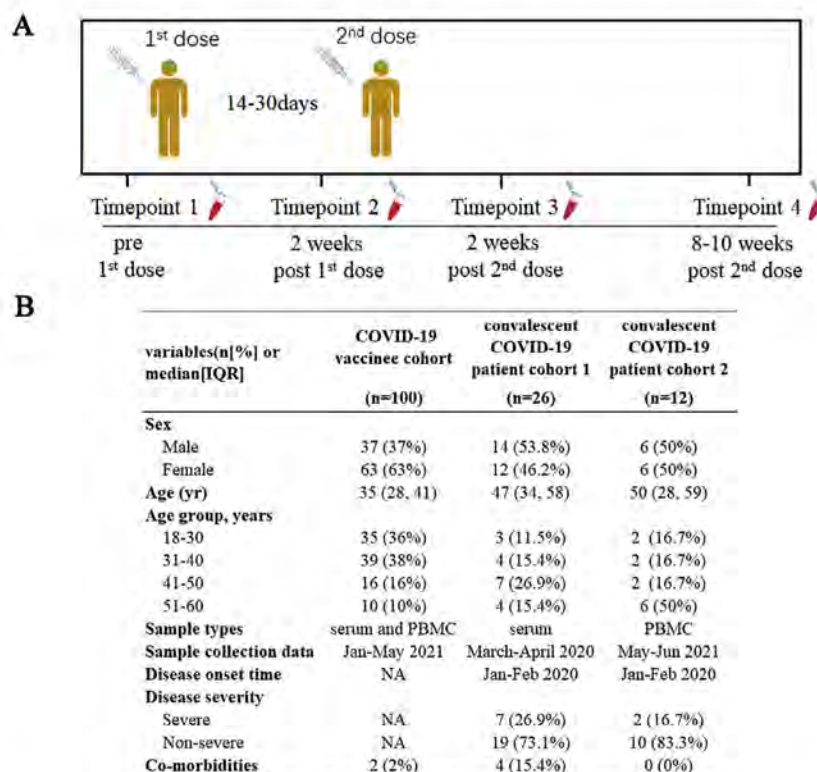


Fig. 1. The study design and the characteristics of participants in our cohort. (A) The study design of our vaccine cohort. (B) The characteristics of three study cohorts used in our study, including the vaccine cohort who received two doses of CoronaVac, the convalescent coronavirus disease 2019 (COVID-19) patient cohort 1 and the convalescent COVID-19 patient cohort 2.

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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).

Immunoglobulin (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 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-

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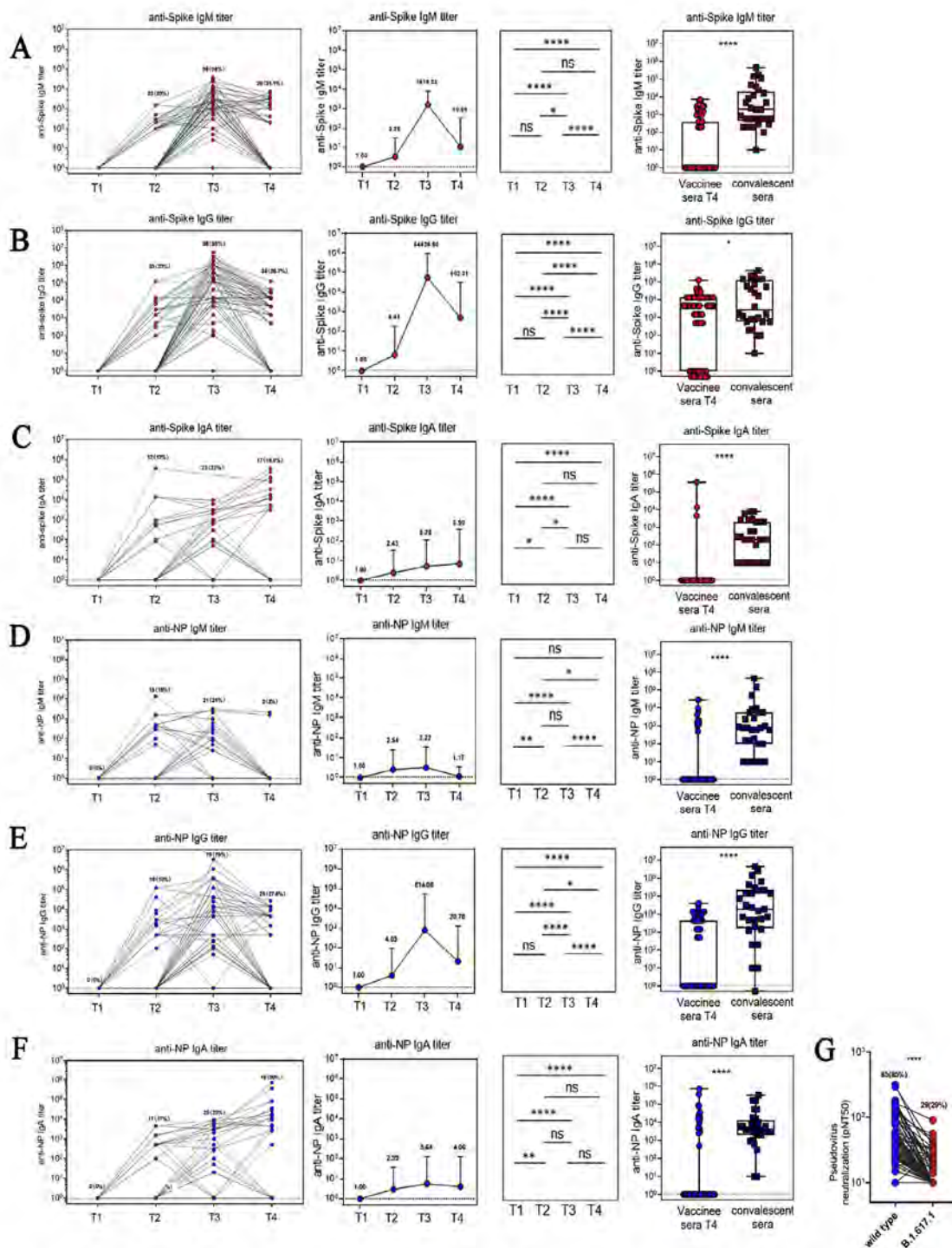


Fig. 2. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific antibody responses following CoronaVac immunization. (A–F) Dynamic antibody titres for (A) anti-spike IgM, (B) anti-spike IgG, (C) anti-spike IgA, (D) anti-NP IgM, (E) anti-NP IgG, and (F) anti-NP IgA at four time points 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.

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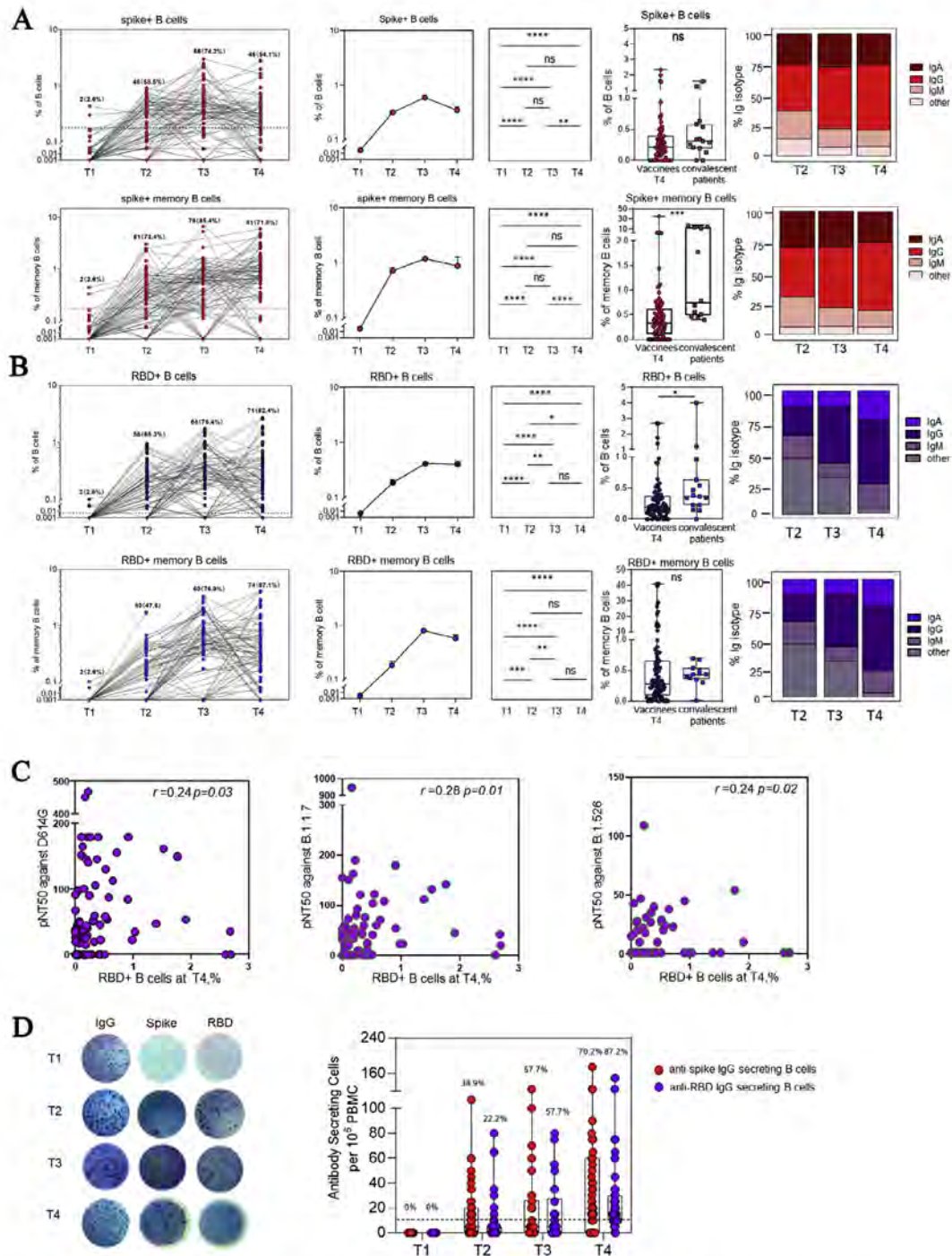


Fig. 3. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific circulating B cell and memory B cell responses following CoronaVac immunization. (A) Frequency of spike⁺ B cells and spike⁺ memory B cells over time in vaccinees. Frequency of spike⁺ B cells or memory spike⁺ B cells 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.

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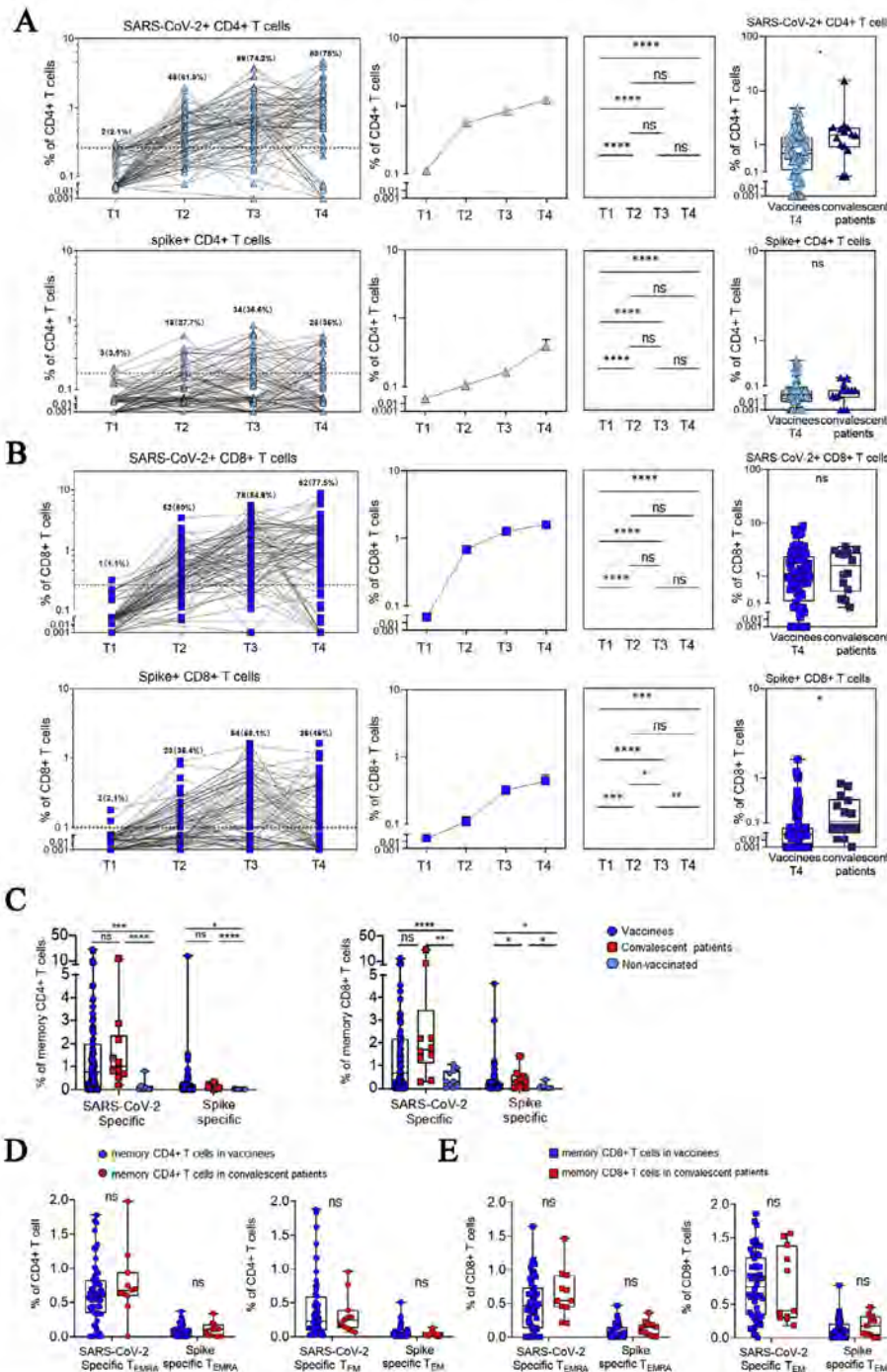


Fig. 4. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific circulating CD4⁺ and CD8⁺ T cell responses following CoronaVac immunization. (A) Frequency of SARS-CoV-2-specific (top) and spike-specific (bottom) CD4⁺ T cells over time among vaccinees, the magnitude of which at T4 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.

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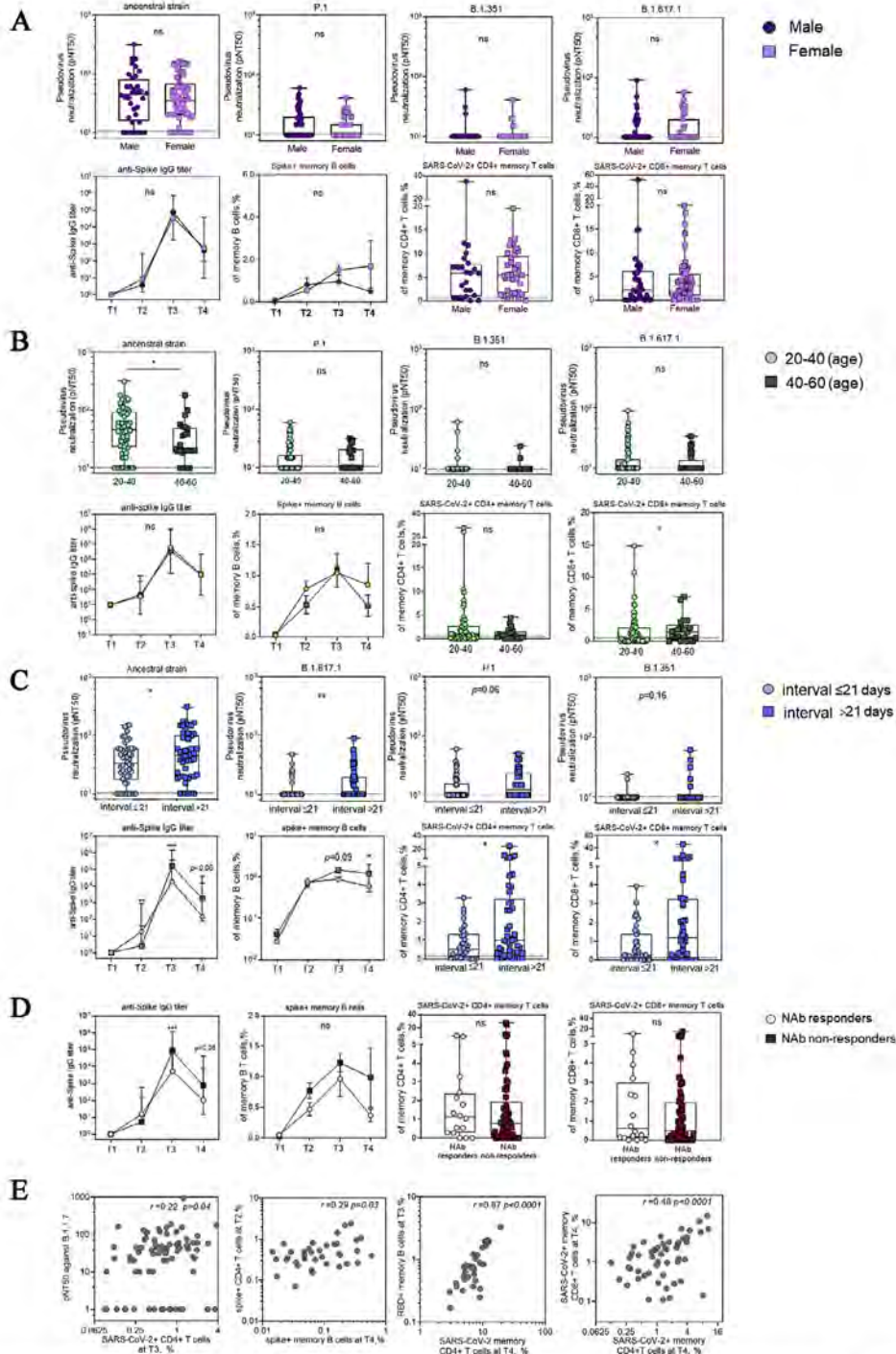


Fig. 5. Association of various factors with vaccine-elicited adaptive responses. (A-C) Serum titres that achieved 50% pseudovirus neutralization (pNT50) against the ancestral strain, the P.1, the B.1.351, the B.1.17.1, anti-spike IgG titre, the frequency of spike-specific memory B cells, the frequency 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 memory CD4⁺ T 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-specific 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.

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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 spike-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 adaptive 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-LCY-PY-10), Nanjing Medical Science and Technique Development Foundation (QRX17141, YKK19056, YKK20058 and YKK20076), National Natural Science Foundation of China (82002133), and

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2021.10.006>.

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1.11. CoronaVac induces rapid and durable antibody responses for up to 12 months, study says

A scientific study published by Chinese researchers from the Chinese Center for Disease Control and Prevention and from the Capital Medical University, both in Beijing, shows that CoronaVac induces a humoral and cellular immune response that remains in the body for one year. The article was submitted to the prestigious British medical publication *The Lancet*, and was published as a preprint.

A total of 150 volunteers were analyzed, aged between 18 and 59 years old, who received the two doses of the vaccine with an interval of 14 days. In order to follow the evolution of the immunological status of the participants, blood samples were collected before the first dose, and also one, three, six and twelve months after the second dose.

Scientists found that one month after the complete immunization, binding antibodies and neutralizing antibodies appeared rapidly. The seropositive rate of binding antibodies was 99% and the neutralizing antibody rate was 50%. From the third to the 12th month

after immunization, there was a slight decrease in the neutralizing antibodies and binding antibodies. In the 12th month, however, the antibodies were still detectable.

In more technical terms, secretion of interferon-gamma (IFN- γ) and interleukin 2 (IL-2) specifically induced by RBD (receptor binding domain) persisted at high levels for up to six months, and could be observed throughout the 12-month analysis. In addition, SARS-CoV-2-specific CD4 + TCM, CD4 + TEM, CD8 + TEM and CD8 + TE cells were all detectable and functional for up to 12 months after administration of the second dose.

Thus, the Chinese researchers have found the persistence of the immune response induced by CoronaVac in a 2-dose regimen. It was proven that the vaccine not only induced durable binding and neutralizing antibody responses, but also SARS-CoV-2-specific memory CD4+ and CD8+ T cells for up to 12 months.

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**Status of Humoral and Cellular Immune Responses within 12 months Following
CoronaVac Vaccination against COVID-19**

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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.

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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 titer 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 monitored 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 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.

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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 use² 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 China⁴, 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 (Beijing, 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 titres 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 assay⁶. All procedures related to virus neutralisation test were performed in a level 3 biosafety laboratory from Sinovac

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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 FACSLytic™, 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 immunogenicity 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 presented 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, paired 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 figure 1.

Chemiluminescent immunoassay (CLIA) showed that at baseline, none of the participants had any detectable RBD-specific IgG antibody (figure 2). At 1 month after the second vaccination, titers of RBD-specific IgG antibodies were strikingly enhanced

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to a maximum S/CO value of 11.26 (95% confidence interval [CI], 9.29 to 13.24), and the seropositive rate 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 1 month, 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 sera of all study participants at baseline (figure 2). At 1 month 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% (71 of 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

frequency (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 detectable at each time point after vaccination.

Memory T-cell subsets, expression of IFN- γ , IL-2, and GrzB were analyzed by using 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, representing 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). Conversely, 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

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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 able to elicit durable SARS-CoV-2-specific memory CD4⁺ T cells, but also SARS-CoV-2-specific memory CD8⁺ T cells.

Discussion

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 results⁴ and showed that SARS-CoV-2 RBD-specific binding and neutralisation antibody responses to immunization with CoronaVac decreased gradually with time, 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 monitored 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 of 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 observed 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 receiving a single dose of 5×10^{10} viral particles

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booster vaccine.¹⁵ Numerically, the humoral responses of CoronaVac are not as strong as other COVID-19 vaccines, however, we should bear that in our mind, i.e., it is difficult to directly evaluate the capacities 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 currently 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 a crucial role in defending 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 temporary, 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 reported 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 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 predominately 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-

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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.

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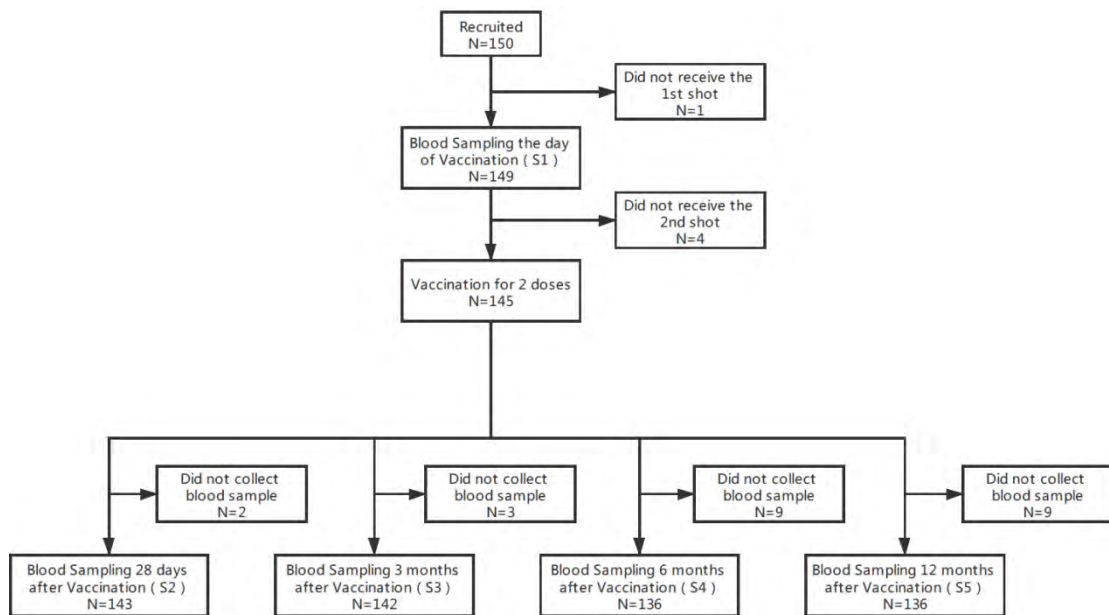


Figure 1: Design and Schedule of samples collection.

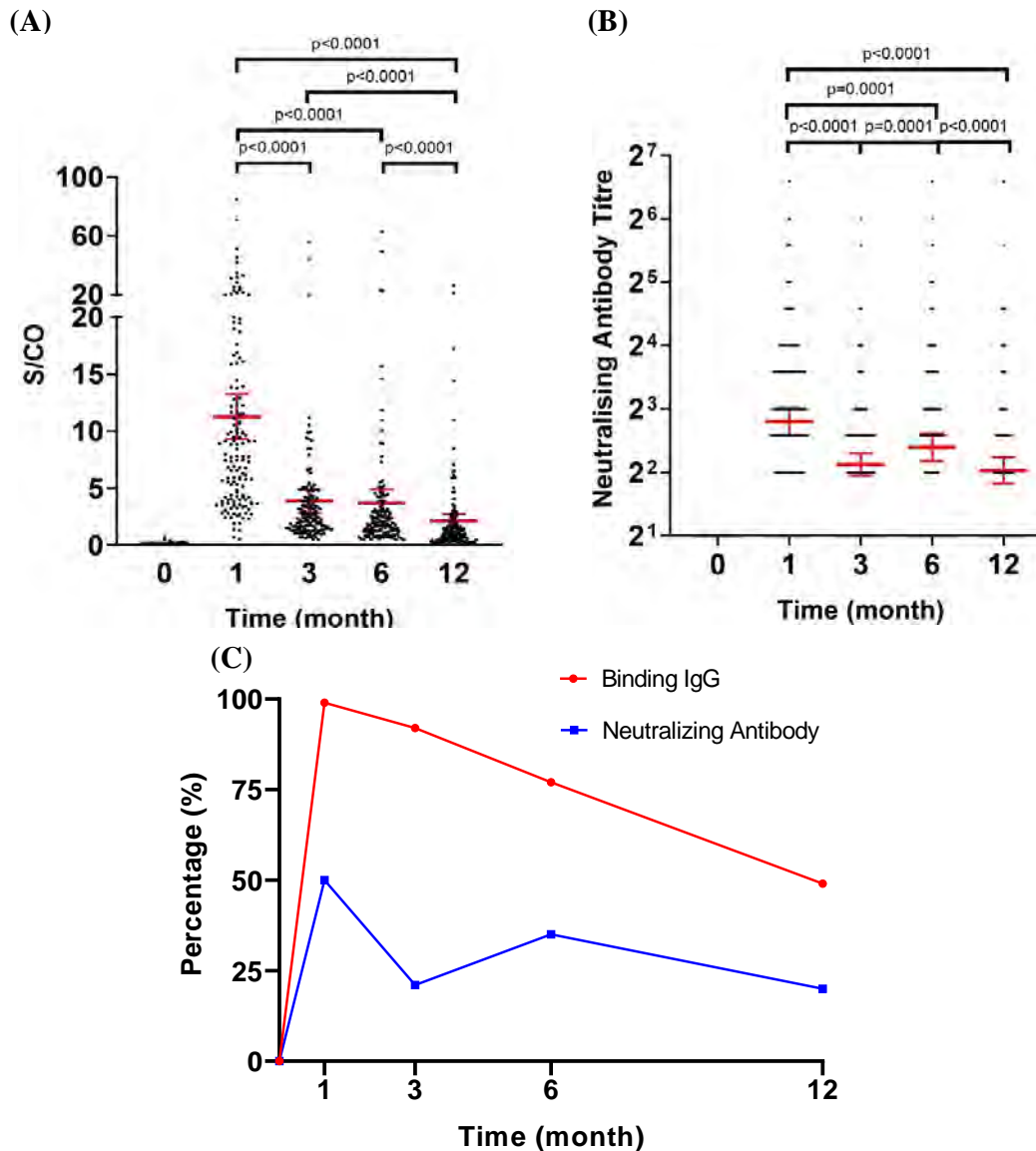


Figure 2: Status of sera IgG and neutralising antibody response following CoronaVac vaccination.

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

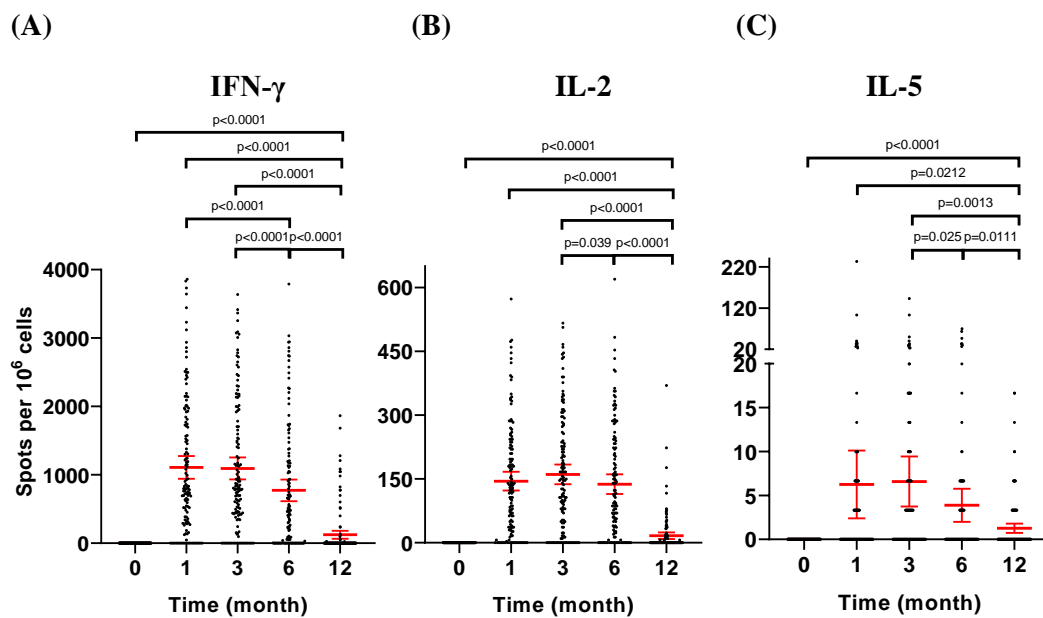


Figure 3: Status of specific T-cell responses following CoronaVac vaccination.

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.

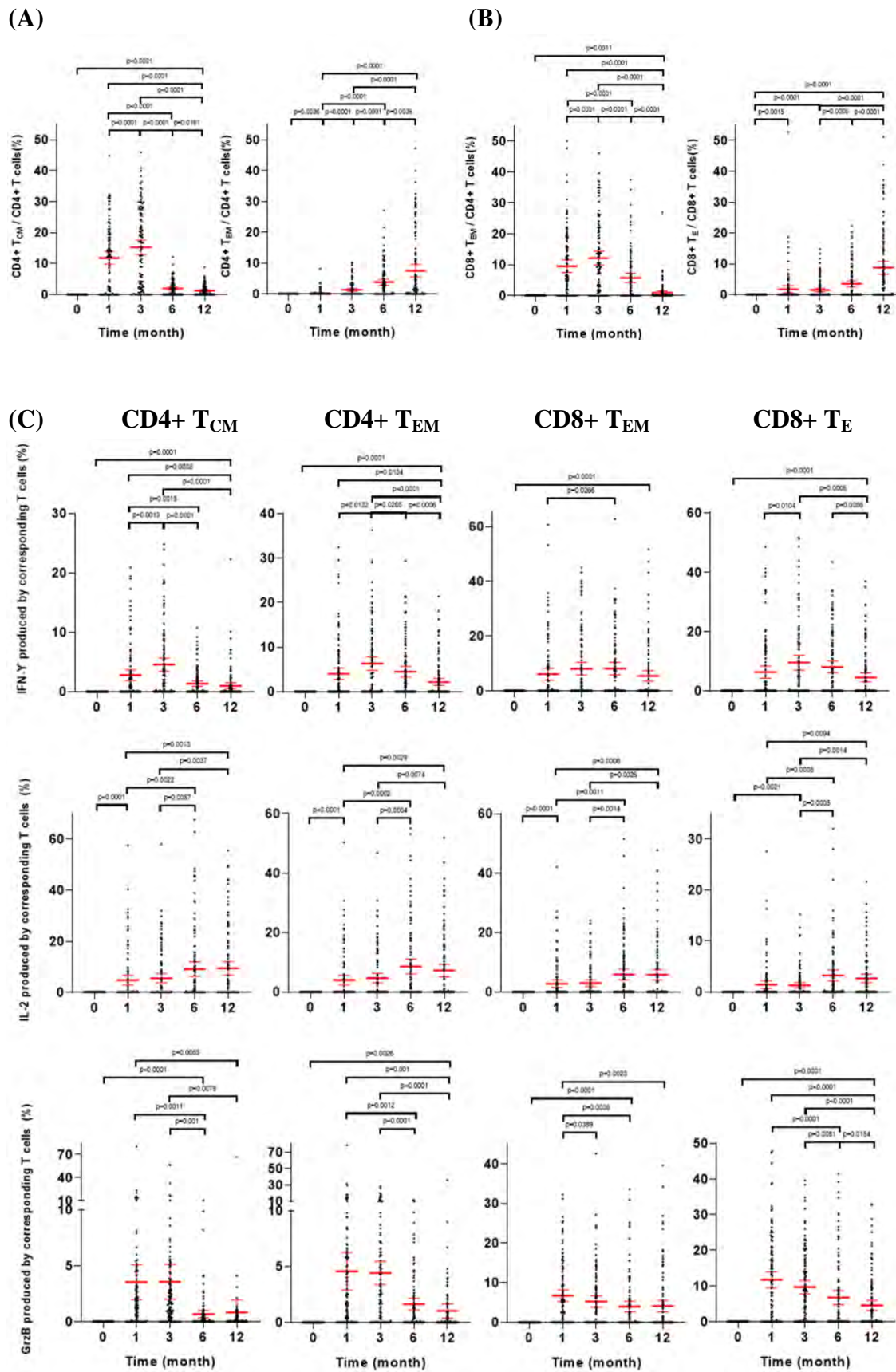


Figure 4 : Status of distribution and expression of cytokines by T_{CM} and T_{EM}

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 induces a high production of neutralizing antibodies, reveals brazilian study

A study published in *Vaccines* journal by researchers from the State University of Pará (UEPA) and the Federal University of Pará (UFPA), in Brazil, demonstrated that CoronaVac induces the production of antibodies capable of neutralizing SARS-CoV-2 on more than 70% of the immunized, reaching 93% in individuals between 21 and 40 years old.

The scientists analyzed the serum of 358 residents in Belém, on Pará, aged between 21 and 96 - 138 men and 220 women. All of them were vaccinated with both doses of CoronaVac with a 20-day interval and blood samples were collected between March and April of 2021.

From the participants, 205 tested to evaluate the total of antibodies against SARS-CoV-2. Of those, 77,6% presented seropositivity. The other 153 individuals tested the presence of neutralizing antibodies specific

for the receptor-binding domain (RBD) and 72,6% had positive results.

The titers of neutralizing antibodies were significantly higher on younger individuals - 93% among participants between 21 and 40 years, 76% among 41 and 60 and 72% among 61 and 80 - which may be associated with the senescence of the immune system, according to researchers. However, besides the presence of antibodies, the immunity is also associated with the response of memory T and B cells that may not be detected by the serological tests. Other studies have already shown that the Butantan vaccine induces significant response from those cells, responsible for detecting the presence of the virus and quickly react with the activation of the defense cells and the production of antibodies.

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Communication

Assessment of Anti-SARS-CoV-2 Antibodies Post-Coronavac Vaccination in the Amazon Region of Brazil

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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

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 ≥ 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 ≥ x ≤ 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-reactive, 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-reactive) 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.

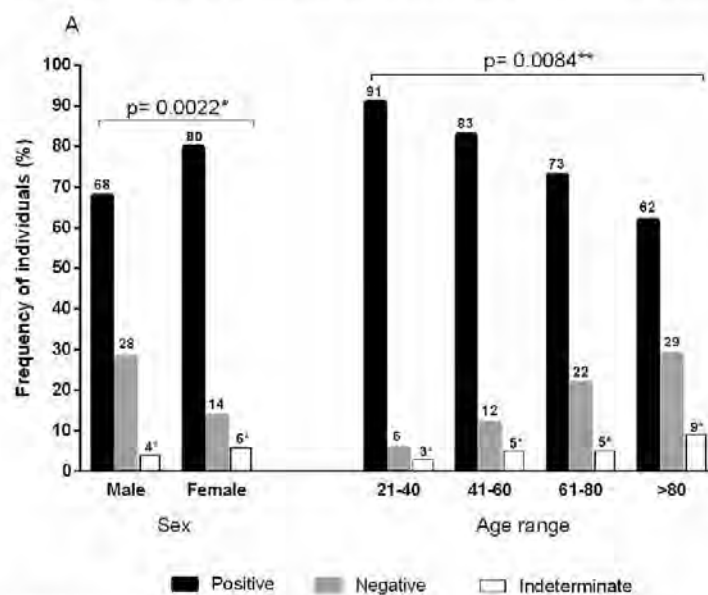


Figure 1. Cont.

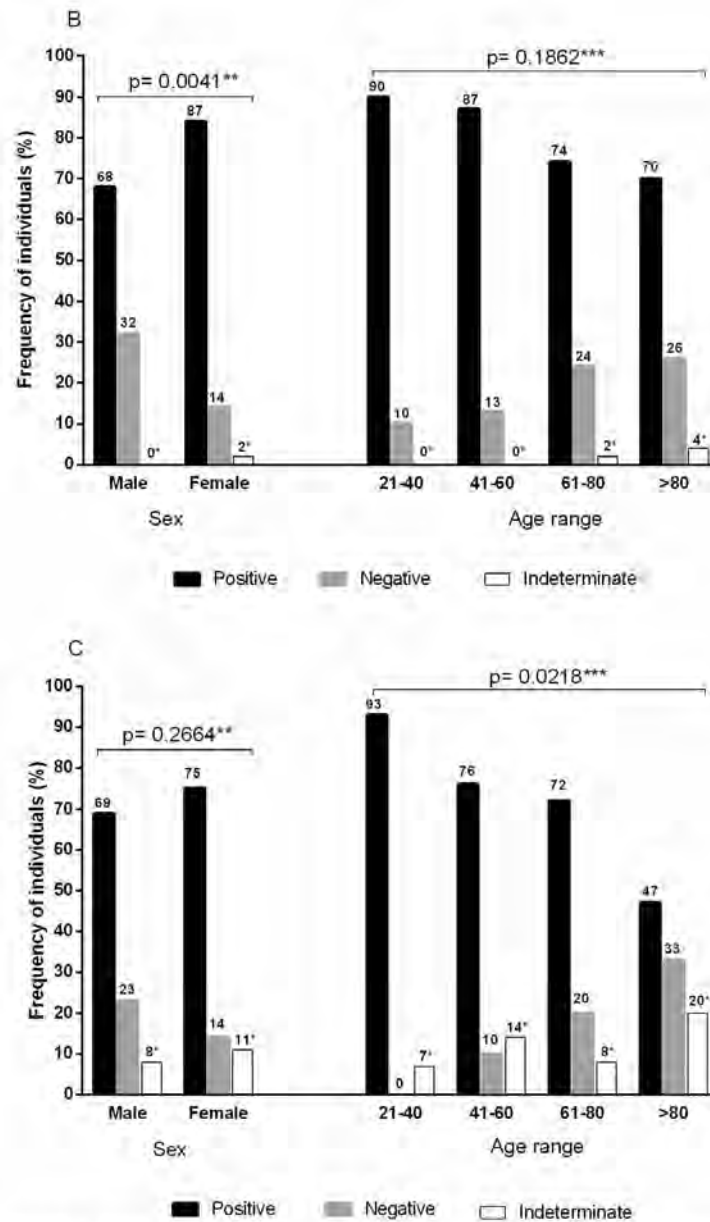


Figure 1. Frequencies of anti-SARS-CoV-2 antibodies according to sex and age group. (A) Pooled frequencies of anti-SARS-CoV-2 total antibodies (S1/S2) plus anti-SARS-CoV-2 IgG neutralizing antibodies (RBD-S1). Sample size by sex: male ($n = 138$) and female ($n = 220$). Sample size by age group: 21–40 ($n = 35$), 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.

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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.

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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.

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1.13. CoronaVac produces antibodies against Covid-19 on 87% of the vaccinated individuals in Indonesia

CoronaVac, Butantan's and Sinovac's vaccine against Covid-19, produced antibodies against SARS-CoV-2 in 87,15% of the immunized 28 days after the second dose, according to a study made with millions of people in Indonesia.

This is the result of a phase 3 clinical trial conducted by scientists of the Medicine School of the Universitas Padjadjaran, in Bandung, and by the Ministry of Health from Indonesia, published in Vaccine journal in September 2021.

The randomized, double-blinded and placebo-controlled clinical trial included a total of 1.620 healthy adults with age between 18 and 59, randomic divided among those that received both doses or placebo, in the months of August, September and October of 2020.

For those that did receive both doses, the efficacy of CoronaVac was 65,30% - a high efficacy that follows the pattern shown in studies conducted in other countries, such as Turkey, Chile and Brazil.

CoronaVac prevented severe cases and deaths

During the period of vigilance of the study, there were 49 cases of Covid-19 among the volunteers. From those, seven immunized and 18 cases in the placebo group were symptomatic and occurred in a period between 14 days and three months after the second dose. There weren't any reports of severe cases or deaths by Covid-19 among the participants of the study.

For safety evaluation, the adverse events requested and non requested were collected after the first and second vaccination in 14 and 28 days, respectively. Blood samples were collected for a trial of antibodies before and 14 days after the second dose.

The majority of the adverse reactions were classified as mild and the most reported was pain in the area of the injection.

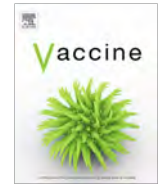
Of the 1.620 participants, 1.046 were male (64,57%) and 574 were female (35,43%).

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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

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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.

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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.

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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

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 $\geq 37.5^{\circ}\text{C}$), 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 $\mu\text{g}/0.5\text{ mL}$ 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 $\mu\text{g}/0.5\text{ mL}$ 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 $+2^{\circ}\text{C}$ to $+8^{\circ}\text{C}$.

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 μ l aliquot of the specimen was added to 560 μ l 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°C 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 Institute of Health Research & 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 ($\geq 39.0^{\circ}\text{C}$). 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 comparison 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 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 ± 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

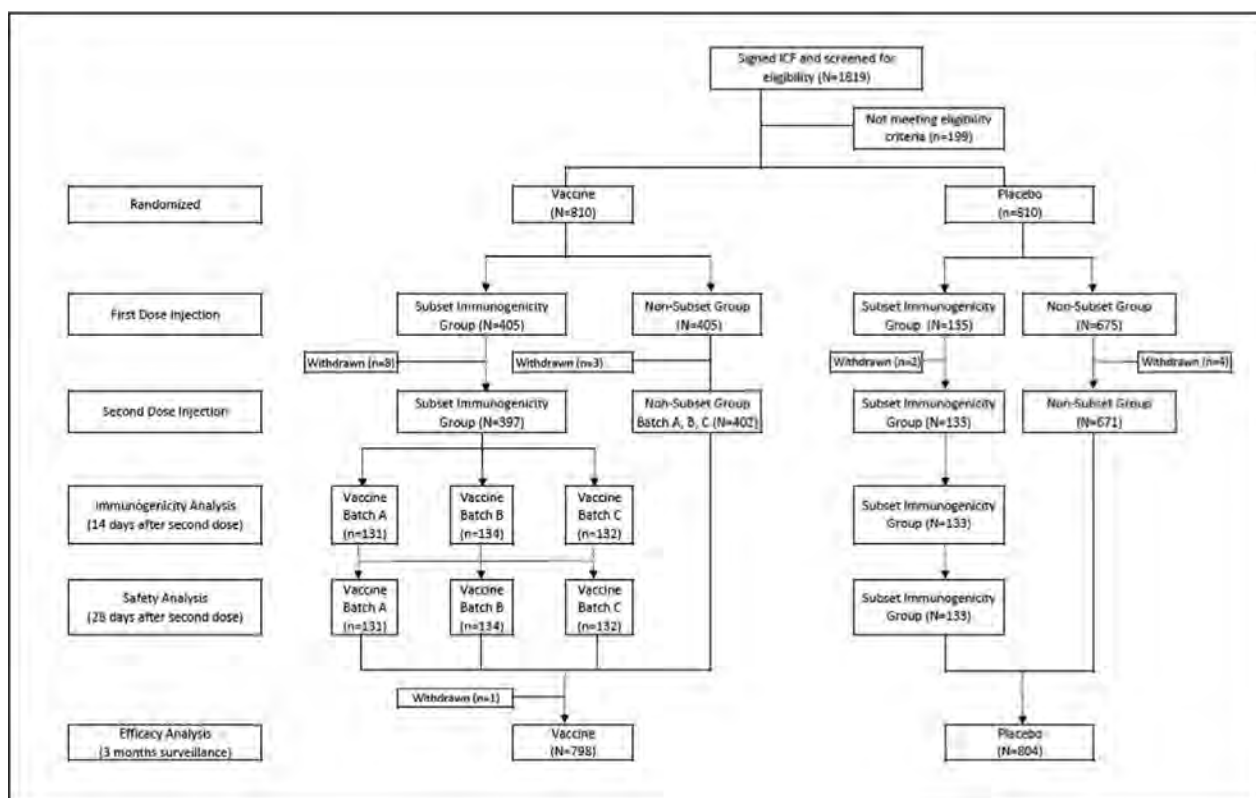


Fig. 1. Participant Disposition.

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/m ²)	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.

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

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

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.

Endpoint	Vaccine			Placebo			Vaccine Efficacy (%)
	No. of cases	Mean follow-up days	Incidence rate (per 100 person years)	No. of cases	Mean follow-up days	Incidence rate (per 100 person years)	
Symptomatic confirmed laboratory cases COVID-19 starting 14 days after second injection	7	80.78	3.904	18	72.08	11.25	65.30%
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(%) (95% CI)	44 (11.08) (8.36–14.55)	14 (10.53) (6.37–16.89)	0.859**
		GMT [†]	220.27	220.37	0.990****
		(95% CI) Median	(212.87–227.93) 200.00	(206.45–235.24) 200.00	
	V3	Seropositive rate n(%) (95% CI)	396 (99.74) (99.26–100)	7 (5.29) (1.47–9.06)	<0.001**
		Seroconversion n(%) (95% CI)	387 (97.48) (95.43–98.63)	1 (0.75) (0.13–4.14)	< 0.001**
		GMT [†] (95% CI) Median	5181.19 (4746.13–5656.14) 5333.35	223.61 (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(%) (95% CI)	380 (95.72) (93.25–97.31)	1 (0.75) (0.13–4.14)	<0.001**
		Seroconversion n (%) (95% CI)	346 (87.15) (83.50–90.09)	0 (0.00) (0–2.81)	< 0.001**
		GMT [†] (95% CI) Median	15.76 (14.57–17.04)	2.02 (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	0.898***
		(95% CI)	(4369.78–5937.59)	(4656.29–6312.77)	(4314.30–5869.76)	
	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.

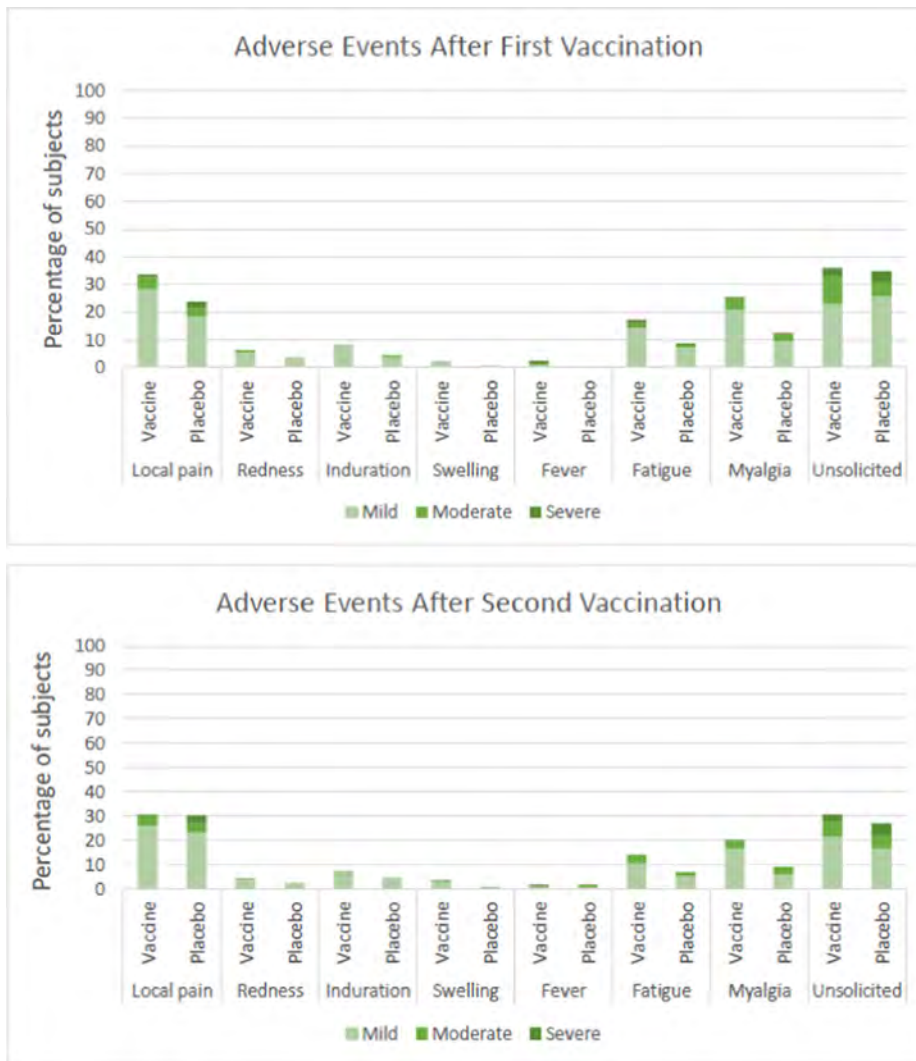


Fig. 2. Adverse Events occurring after the First and Second Vaccine Injection.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2021.09.052>.

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1.14. CoronaVac promotes high humoral and cellular immune response, Chilean study shows

A Chilean research published in the *Clinical Infectious Diseases* journal has attested the safety and immunogenicity of CoronaVac in healthy adults, showing that the vaccine induces a high cellular and humoral immune response (antibody production). Released in September 2021, the study was conducted by researchers at the Pontificia Universidad Católica de Chile.

A total of 434 volunteers were followed, with 397 aged 18 to 59 and 37 aged over 60. Among the participants, 390 took two doses of the vaccine and 44 received a placebo. No serious adverse effects were reported and the main symptoms were injection site pain and headache.

The humoral immune response was assessed in 81 volunteers. One month after the second dose of the vaccine, the seroconversion rate of IgG antibodies specific for the receptor-binding domain (RBD) of

the SARS-CoV-2 Spike protein was 84.4% for individuals aged 18 to 59 years old and 70.3% for the elderly. It was also detected an increase in circulating neutralizing antibodies.

The scientists also assessed the cellular immune response in 47 participants. A significant T-cell response was detected, characterized by the secretion of interferon-gamma (IFN- γ) - cytokines that activate macrophages, important defense cells of the body.

“Results indicate that CoronaVac is safe and induces robust humoral and cellular responses, producing RBD-specific antibodies with neutralizing capacity and activating T cells,” the study concludes.

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Safety and Immunogenicity of an Inactivated Severe Acute Respiratory Syndrome Coronavirus 2 Vaccine in a Subgroup of Healthy Adults in Chile

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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

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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 4°C 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.

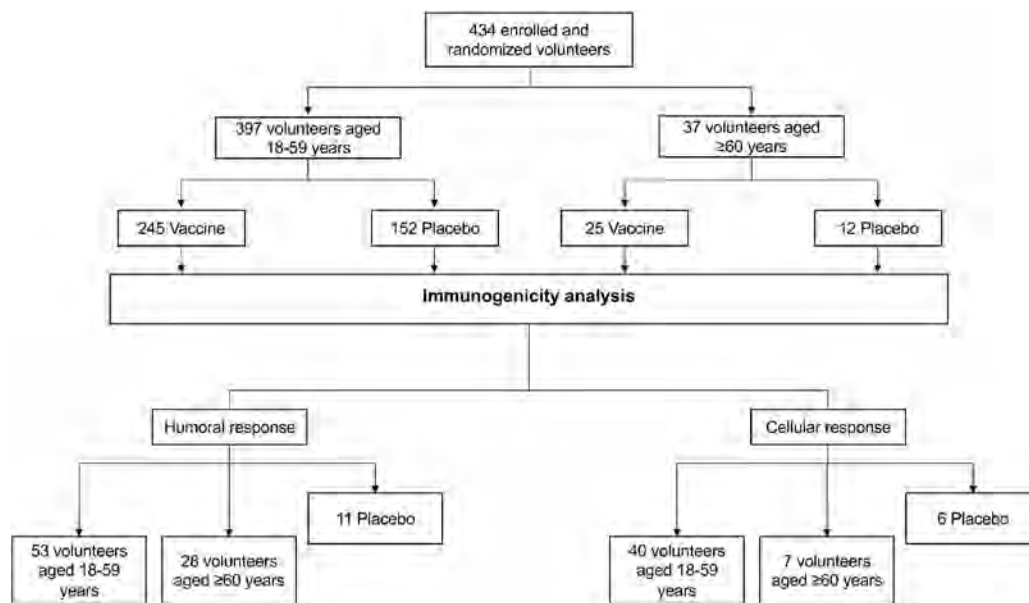


Figure 1. Study profile. Recruitment of volunteers for the phase 3 clinical trial as of February 10, 2021.

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](#).

Adverse Reaction	First Dose (n = 434)		Second Dose (n = 319)		Both Doses (n = 319)		P Value
	Placebo (n = 164)	Vaccine (n = 270)	Placebo (n = 80) ^a	Vaccine (n = 239)	Placebo (n = 80)	Vaccine (n = 239)	
Local reactions							
Pain, n (%) ^b							
<60 y	39 (23.8)	117 (43.3)	16 (20.0)	73 (30.5)	32 (40.0)	133 (55.6)	.015
≥60 y	37 (24.3)	113 (46.1)	15 (20.8)	68 (31.8)	30 (41.7)	125 (58.4)	.014
Induration (%)							
<60 y	2 (16.7)	4 (16.0)	1 (12.5)	5 (20.0)	2 (25.0)	8 (32.0)	.999
≥60 y	1 (0.6)	8 (3.0)	0 (0.0)	15 (6.3)	0 (0.0)	21 (8.8)	.015
Swelling (%)							
<60 y	1 (0.7)	7 (2.9)	0 (0.0)	13 (6.1)	0 (0.0)	18 (8.4)	.043
≥60 y	0 (0.0)	1 (4.0)	0 (0.0)	2 (8.0)	0 (0.0)	3 (12.0)	.999
Pruritus (%)							
<60 y	4 (2.4)	15 (5.6)	2 (2.5)	6 (2.5)	3 (3.8)	17 (7.1)	.099
≥60 y	4 (2.6)	15 (6.1)	2 (2.8)	6 (2.8)	3 (4.2)	17 (7.9)	.099
Erythema (%)							
<60 y	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	...
≥60 y	3 (1.8)	10 (3.7)	2 (2.5)	3 (1.3)	2 (2.5)	10 (4.2)	.602
Swelling (%)							
<60 y	3 (2.0)	10 (4.1)	1 (1.4)	3 (1.4)	1 (1.4)	10 (4.7)	.999
≥60 y	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	1 (12.5)	0 (0.0)	.242
Systemic reactions							
Headache (%)							
<60 y	50 (30.5)	107 (39.6)	15 (18.8)	46 (19.2)	39 (48.8)	116 (48.5)	.922
≥60 y	49 (32.2)	102 (41.6)	12 (16.7)	42 (19.6)	36 (50.0)	109 (50.9)	.891
Fatigue (%)							
<60 y	1 (8.3)	5 (20.0)	3 (37.5)	4 (16.0)	3 (37.5)	7 (28.0)	.32
≥60 y	32 (19.5)	58 (21.5)	10 (12.5)	25 (10.5)	22 (27.5)	64 (26.8)	.613
Myalgia (%)							
<60 y	31 (20.4)	55 (22.4)	8 (11.1)	23 (10.7)	20 (27.8)	60 (28.0)	.932
≥60 y	1 (8.3)	3 (12.0)	2 (25.0)	2 (8.0)	2 (25.0)	4 (16.0)	.241
Diarrhea (%)							
<60 y	23 (14.0)	48 (17.8)	9 (11.3)	19 (7.9)	19 (23.8)	54 (22.6)	.367
≥60 y	22 (14.5)	46 (18.8)	8 (11.1)	16 (7.5)	18 (25.0)	50 (23.4)	.336
Nausea (%)							
<60 y	1 (8.3)	2 (8.0)	1 (12.5)	3 (12.0)	1 (12.5)	4 (16.0)	.999
≥60 y	18 (11.0)	36 (13.3)	5 (6.3)	18 (7.5)	15 (18.8)	44 (18.4)	.701
Arthralgia (%)							
<60 y	17 (11.2)	36 (14.7)	4 (5.6)	16 (7.5)	14 (19.4)	42 (19.6)	.58
≥60 y	1 (8.3)	0 (0.0)	1 (12.5)	2 (8.0)	1 (12.5)	2 (8.0)	.999
Anorexia (%)							
<60 y	18 (11.0)	25 (9.3)	3 (3.8)	9 (3.8)	11 (13.8)	27 (11.3)	.999
≥60 y	18 (11.8)	22 (9.0)	2 (2.8)	9 (4.2)	10 (13.9)	24 (11.2)	.736
Headache (%)							
<60 y	0 (0.0)	3 (12.0)	1 (12.5)	0 (0.0)	1 (12.5)	3 (12.0)	.242
≥60 y	10 (6.1)	14 (5.2)	2 (2.5)	7 (2.9)	7 (8.8)	18 (7.5)	.999
Swelling (%)							
<60 y	10 (6.6)	13 (5.3)	2 (2.8)	6 (2.8)	7 (9.7)	16 (7.5)	.999
≥60 y	0 (0.0)	1 (4.0)	0 (0.0)	1 (4.0)	0 (0.0)	2 (8.0)	.999
Pruritus (%)							
<60 y	10 (6.1)	18 (6.7)	3 (3.8)	3 (1.3)	6 (7.5)	16 (6.7)	.815
≥60 y	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	...

Table 2. Continued

Adverse Reaction	First Dose (n = 434)		Second Dose (n = 319)		Both Doses (n = 319)		P Value
	Placebo (n = 164)	Vaccine (n = 270)	Placebo (n = 80) ^a	Vaccine (n = 239)	Placebo (n = 80)	Vaccine (n = 239)	
<60 y	10 (6.6)	16 (6.5)	2 (2.8)	3 (1.4)	5 (6.9)	14 (6.5)	.999
≥60 y	0 (0.0)	2 (8.0)	1 (12.5)	0 (0.0)	1 (12.5)	2 (8.0)	.999
Pruritus (%)	2 (1.2)	10 (3.7)	0 (0.0)	4 (1.7)	1 (1.3)	14 (5.9)	.127
<60 y	2 (1.3)	9 (3.7)	0 (0.0)	4 (1.9)	1 (1.4)	13 (6.1)	.202
≥60 y	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)	.999
Exanthema (%)	1 (0.6)	7 (2.6)	0 (0.0)	1 (0.4)	1 (1.3)	8 (3.3)	.459
<60 y	1 (0.7)	7 (2.9)	0 (0.0)	1 (0.5)	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)	0 (0.0)	3 (1.3)	0 (0.0)	8 (3.3)	.209
<60 y	0 (0.0)	5 (2.0)	0 (0.0)	2 (0.9)	0 (0.0)	4 (1.9)	.575
≥60 y	1 (8.3)	1 (4.0)	0 (0.0)	1 (4.0)	0 (0.0)	1 (4.0)	.999
Vomiting (%)	3 (1.8)	1 (0.4)	0 (0.0)	4 (1.7)	0 (0.0)	4 (1.7)	.575
<60 y	3 (2.0)	1 (0.4)	0 (0.0)	4 (1.9)	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)	0 (0.0)	0 (0.0)	1 (1.3)	1 (0.4)	.439
<60 y	1 (0.7)	1 (0.4)	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 = 245; vaccine < 60 first dose: n = 12; placebo ≥ 60 second dose: n = 8; vaccine ≥ 60 first dose: n = 25; vaccine ≥ 60 years second dose: n = 25.

^aAs of February 24, 2021, only 80 volunteers from the placebo arm had received their second dose.

^bPercentages were calculated from the total number of volunteers in each group.

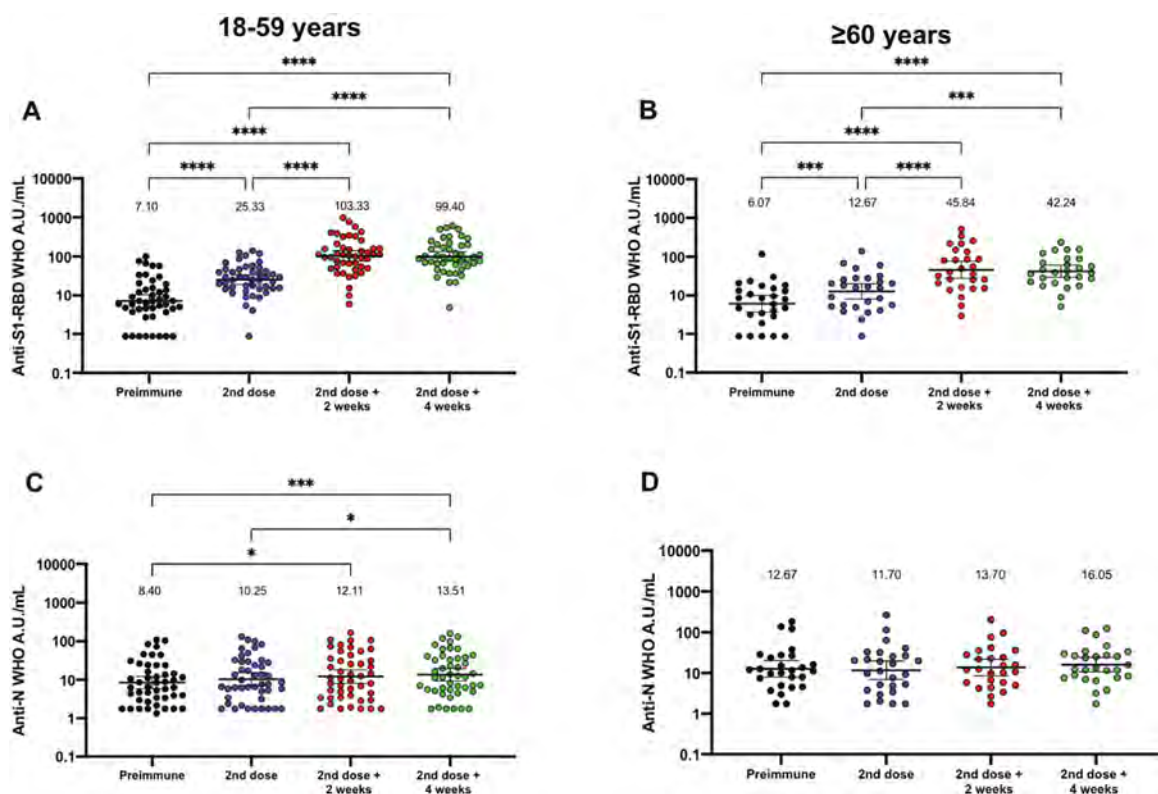


Figure 2. Immunization with CoronaVac induces specific IgG against SARS-CoV-2 antigens in participants aged 18–59 years and ≥ 60 years after 2 immunizations in a 0–14 schedule. Titers of IgG antibodies after 2 doses of CoronaVac were evaluated for immunized participants (excluding seropositive participants 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 log₁₀ 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 (31.94)	54/72 (75.00)	57/72 (79.17)
		GMU (95% CI)	19.60 (15.24–25.22)	76.50 (57.67–101.5)	72.43 (56.96–92.11)
	18–59 years	Seroconversion n/N (%)	18/45 (40.00)	37/45 (82.22)	38/45 (84.44)
		GMU (95% CI)	25.33 (19.07–33.64)	103.33 (75.31–141.8)	99.40 (74.53–132.6)
	≥ 60 years	Seroconversion n/N (%)	5/27 (18.52)	17/27 (62.96)	19/27 (70.37)
		GMU (95% CI)	12.67 (08.03–19.99)	45.84 (27.51–76.36)	42.24 (29.44–60.61)
	Placebo	Seroconversion n/N (%)	0/12 (0)	0/9 (0)	0/0 (N/D)
		GMU (95% CI)	10.43 (04.33–25.10)	6.19 (01.85–20.76)	N/D
Anti-N IgG (WHO A.U./mL)	Total vaccine	Seroconversion n/N (%)	2/72 (2.78)	5/72 (6.94)	7/72 (9.72)
		GMU (95% CI)	10.77 (07.95–14.57)	12.66 (09.36–17.12)	14.4 (10.89–19.04)
	18–59 years	Seroconversion n/N (%)	2/45 (4.44)	5/45 (11.11)	6/45 (13.33)
		GMU (95% CI)	10.25 (06.97–15.08)	12.11 (08.07–18.16)	13.51 (09.21–19.81)
	≥ 60 years	Seroconversion n/N (%)	0/27 (0)	0/27 (0)	1/27 (3.70)
		GMU (95% CI)	11.70 (06.96–19.67)	13.70 (08.60–21.82)	16.05 (10.65–24.18)
	Placebo	Seroconversion n/N (%)	1/12 (8.3)	0/10 (0)	0/0 (-)
		GMU (95% CI)	11.06 (04.03–30.35)	9.61 (02.90–31.90)	N/D

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 S1A and S1B). 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 S1C and S1D). 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.

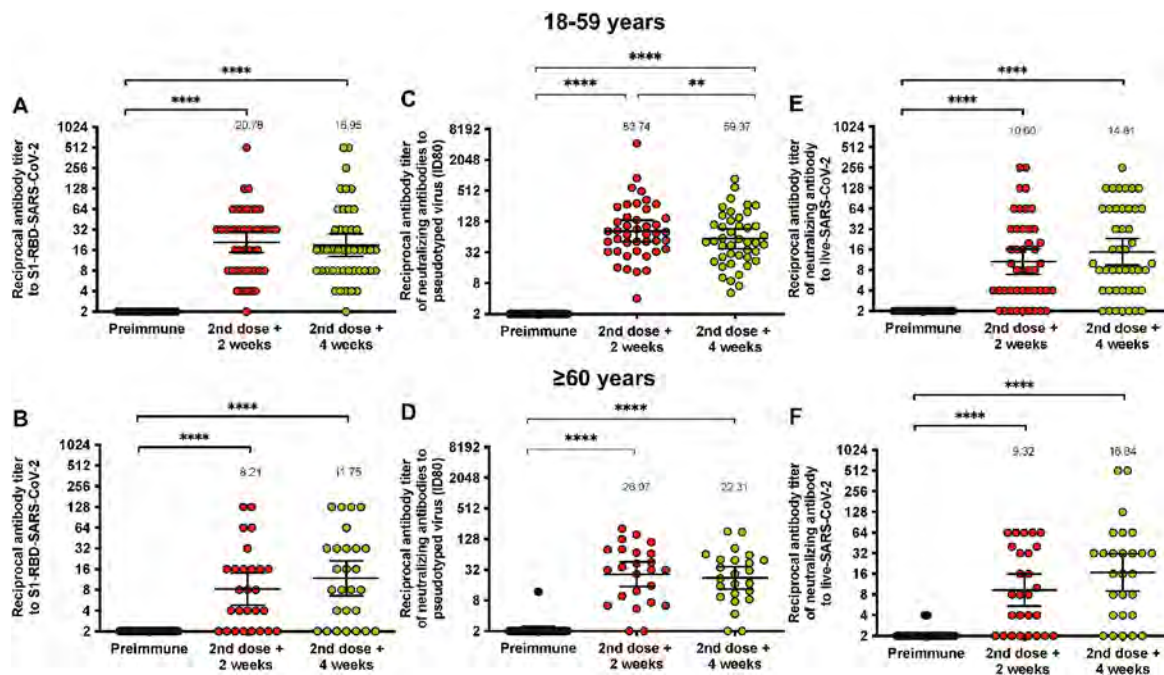


Figure 3. Immunization with CoronaVac induces neutralizing antibodies against SARS-CoV-2 in participants aged 18–59 years and ≥60 years after 2 immunizations in a 0–14 schedule. (A–B) Neutralizing antibody titers were evaluated with a surrogate virus neutralization assay, which quantifies the interaction 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).

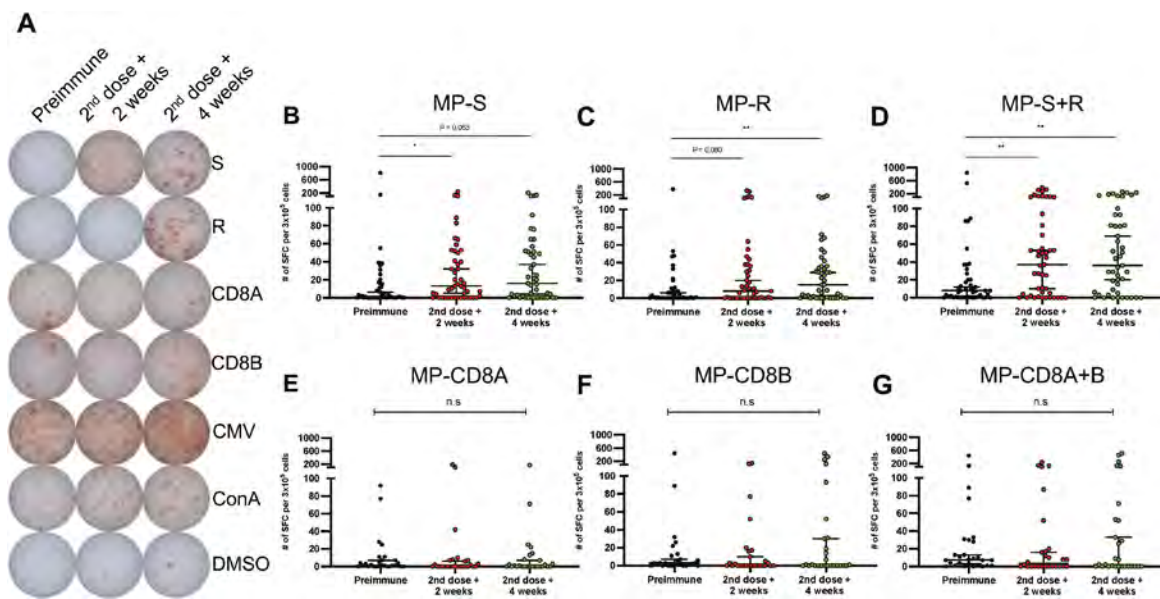


Figure 4. Evaluation of cellular immune response through ELISPOT upon stimulation with Mega Pools of peptides derived from SARS-CoV-2 proteins in volunteers immunized with CoronaVac. Numbers of IFN- γ -secreting cells, determined through ELISPOT as spot forming cells (SFCs) were determined. (A) Representative 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 \pm 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

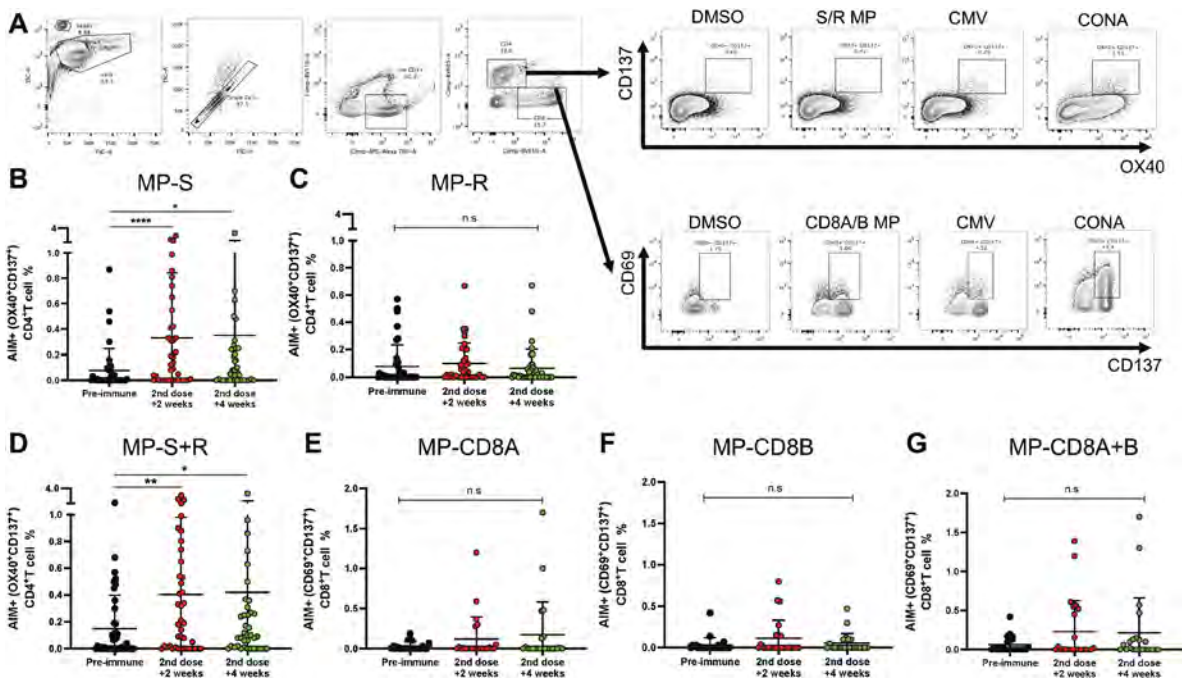


Figure 5. Changes in activation-induced markers (AIMs) expression in T cells through flow cytometry upon stimulation with Mega Pools of peptides derived from SARS-CoV-2 in volunteers immunized with CoronaVac. (A) The gating strategy used to evaluate changes in the expression of AIMs upon stimulation 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

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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.

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1.15. CoronaVac duplicates neutralizing antibodies and increases IgG 4,4 times on those previously infected with Covid-19

A study conducted by researchers of the Medical University of Chongqing, in China, with 85 recovered patients from Covid-19 indicates that CoronaVac is capable of duplicating the amount of neutralizing antibodies and multiplying in 4,4 times the level of IgG antibodies in those previously infected. The preliminary results were disclosed at the Cell Discovery, a publication of the britannic group Nature, in the article Humoral responses in naive or SARS-CoV-2 experienced individuals vaccinated with an inactivated vaccine.

The participants were between three and 84 years old and were previously infected by SARS-CoV-2 in the beginning of 2020. The researchers measured the levels of IgG antibodies and of neutralizing antibodies in the convalescent patients and selected the five that presented individually the lowest indicator at the end of 12 months. They received two doses of CoronaVac with a 21-day interval.

The level of neutralizing antibodies (that protect against an eventual reinfection by the SARS-CoV-2) among the convalescent patients, which was 36 one day before the first dose, increased to 108 two weeks after the second dose. In the control group, that indicator reached 56,

meaning that the amount of neutralizing antibodies generated by the vaccine on those that already had Covid-19 was doubled in comparison to those that weren't infected before.

Among the convalescents, the level of IgG antibodies, which were of 3,68 one day before the vaccination, increased to 47,74 two weeks after the second dose of CoronaVac. It's 4,4 times above the level of 10,81 detected in the control group. The IgG is related to the humoral immunity, which is critical for the combat against SARS-CoV-2 and also performs a fundamental role in the prevention of viral reinfection.

During the 12 months of follow-up, the levels of neutralizing antibodies decreased from 631 in the end of the first month to 84 in the last month. In the case of IgG, the indicator decreased from 28,6 to 7,2 during the same period.

The results suggest that CoronaVac stimulates the humoral memory of the convalescent patients, accelerating the production of neutralizing antibodies and its level of circulation in the blood flow.

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CORRESPONDENCE

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Humoral responses in naive or SARS-CoV-2 experienced individuals vaccinated with an inactivated vaccine

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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 viruses⁶. 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

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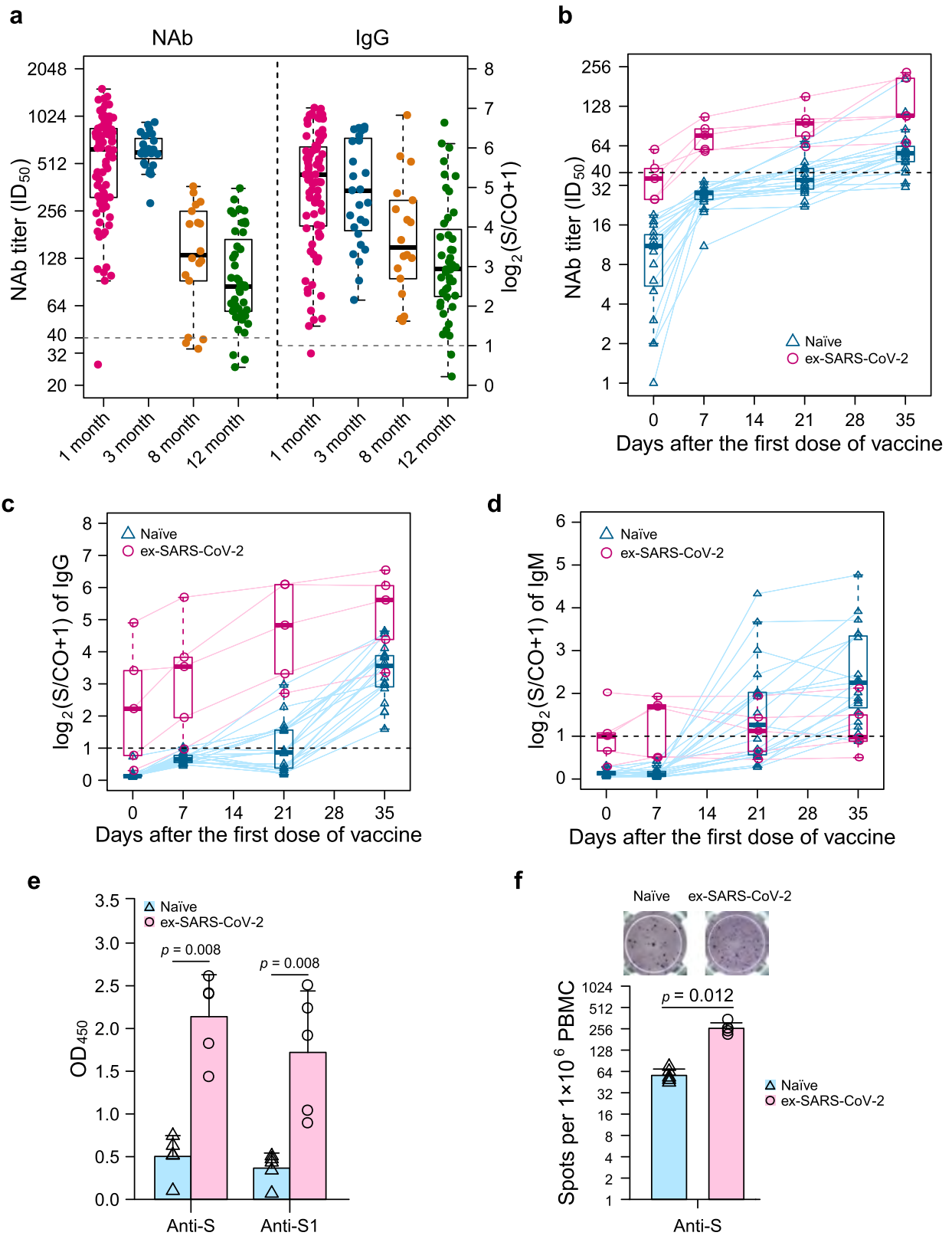


Fig. 1 (See legend on next page.)

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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 (ID₅₀) 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 detect 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 $\times 1.5$ interquartile range (IQR) of antibody levels. In **e, f**, boxes and error bars denote mean \pm 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 NAb 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. NAb were detectable 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 NAb 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 persons⁷.

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.

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Conflict of interest

The authors declare no competing interests.

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1.16. CoronaVac is safe and has an efficacy of 83,5%, according to Turkish clinical study

As it has been confirmed before by the phase 3 clinical trials conducted during 2020 in Brazil to evaluate the efficacy of CoronaVac, a study from the Hacettepe University, in Turkey, demonstrated that Butantan's and Sinovac's vaccine is 83,5% efficient against SARS-CoV-2, besides being safe and well tolerated by the body. The research was published in *The Lancet* and in the National Library of Medicine of the United States.

The phase 3 study, randomized and double blinded, had 10.218 volunteers and was conducted between 14th of December, 2020 and 5th of January, 2021. The participants were assessed seven, 14 and 28 days after receiving each dose of the vaccine. During the follow-up of about 43 days, nine symptomatic cases of Covid-19 were confirmed in the group that took the vaccine and 32 cases were reported in the group that had received the placebo. There were no deaths in neither of the groups.

Besides, CoronaVac induced antibody production in 89,7% of the volunteers. From those, 92% also produced protector levels of neutralizing antibodies at least 14 days after the second dose of the vaccine.

The article also highlighted that the vaccine demonstrated a good profile of safety, without severe adverse events during the period of the study. The majority of the adverse effects was of level 1 and occurred up to seven days after the injection. The total incidence was low (18,9%) and the main symptom was fatigue.

“Our results show that CoronaVac has a good efficacy against the symptomatic infection by SARS-CoV-2 and severe Covid-19 with a very good safety profile in a population between 18 and 59 years old”, said the authors of the article. “The tolerability of CoronaVac was excellent and the incidence of adverse events was low.”

Volunteers from different risk groups and occupations participated in the study, which made the results really close to the context of the real world. A total of 6.646 people received the vaccine, being 3.568 volunteers that received placebos (substance or treatment without an active drug ingredient, like an injection of saline solution). From the total of participants, 57,8% were male and 42,24% were female, all of them between 18 and 59 years old. 3.675 were healthcare workers and 1.463 were obese. And among all the participants, 6.217 had some kind of comorbidity - the majority reported having arterial hypertension.

The phase 3 clinical trial conducted in Brazil by Instituto Butantan involved 16 scientific research centers in seven states and in the Federal District. The study had 12,5 thousand healthcare workers participants, and obtained 62,3% of global efficacy in low, mild and severe cases, in a 21-day interval between doses.

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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



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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 11303 volunteers screened between Sept 14, 2020, and Jan 5, 2021, 10218 were randomly allocated. After exclusion of four participants from the vaccine group because of protocol deviations, the intention-to-treat group consisted of 10214 participants (6646 [65.1%] in the vaccine group and 3568 [34.9%] in the placebo group) and the per protocol group consisted of 10029 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.

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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

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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.

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

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.

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 (Bioeksan, 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 μ g 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

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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.

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

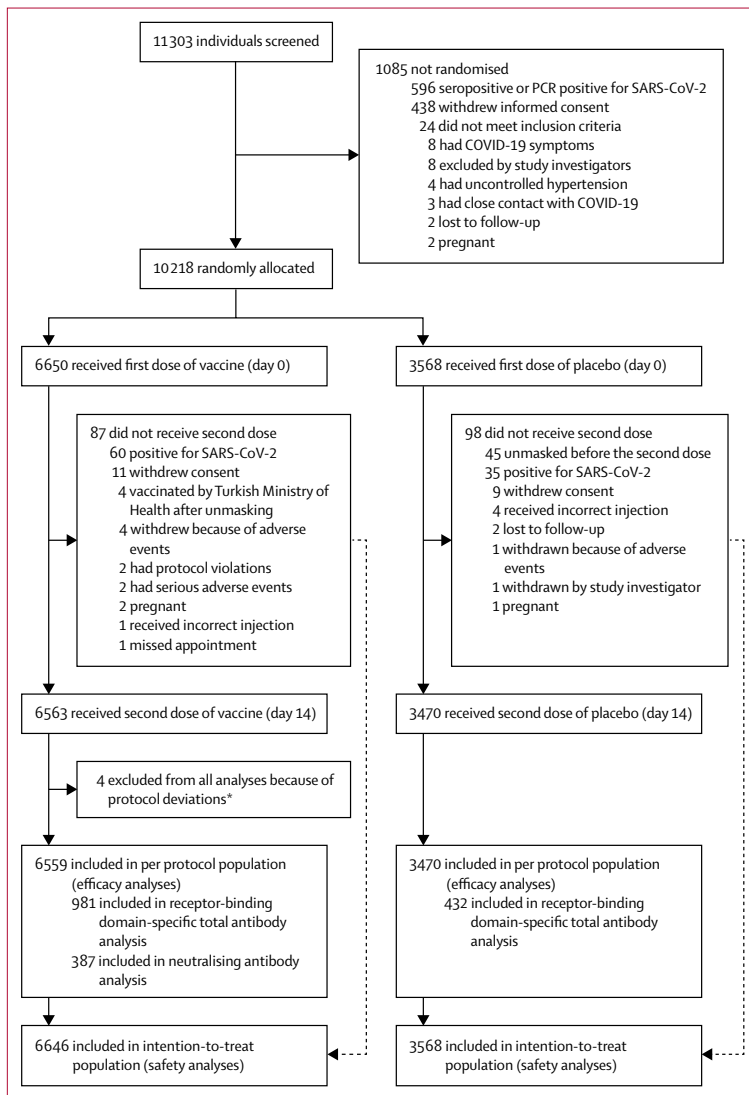


Figure 1: Trial profile
*Four participants in the vaccine group received two doses of the study product; however, because they were older than 59 years on the day of randomisation, they were excluded from all safety and efficacy analyses due to protocol violation.

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

11303 volunteers were screened for eligibility, and 10218 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/m ²		
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).

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 SN₅₀s 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

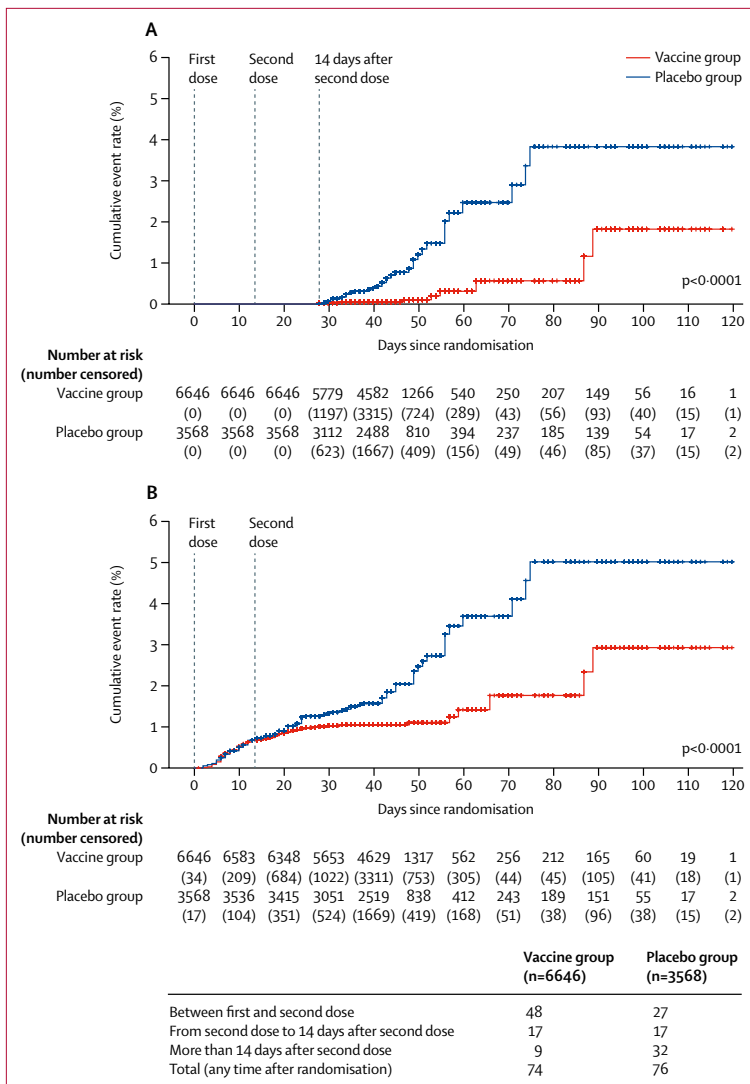


Figure 2: Cumulative incidence curves for COVID-19 cases (A) Cumulative incidence of COVID-19 in the per protocol population (assessed by analysing cases occurring 14 days or more after the second dose of vaccination). (B) Cumulative incidence of COVID-19 in the intention-to-treat population (starting immediately after randomisation).

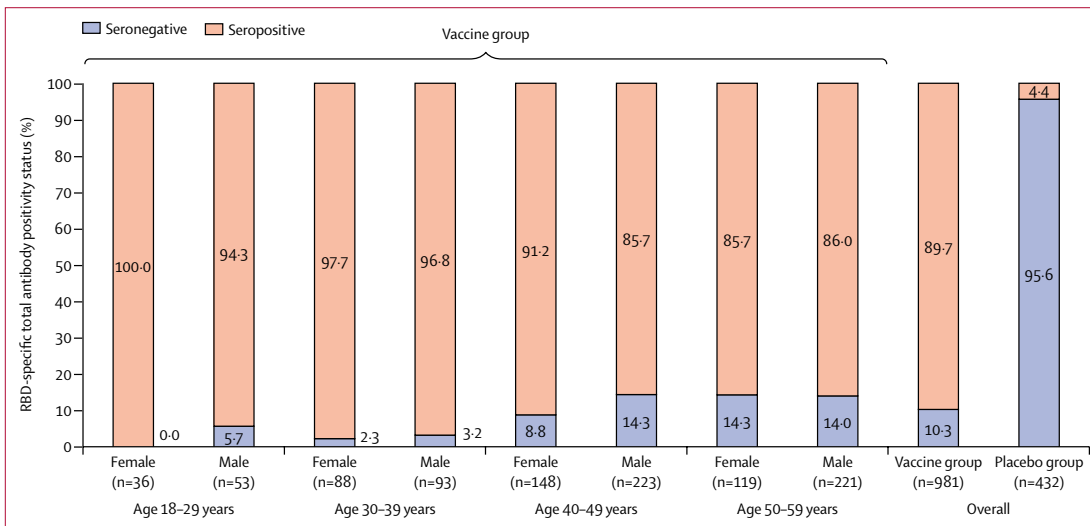


Figure 3: Seropositivity of RBD-specific total antibodies in the vaccine and placebo groups 14 days after the second dose, by age and sex
 The participants with positive RBD-specific antibodies in the placebo group neither reported any symptoms during the follow-up nor had a laboratory confirmed diagnosis 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

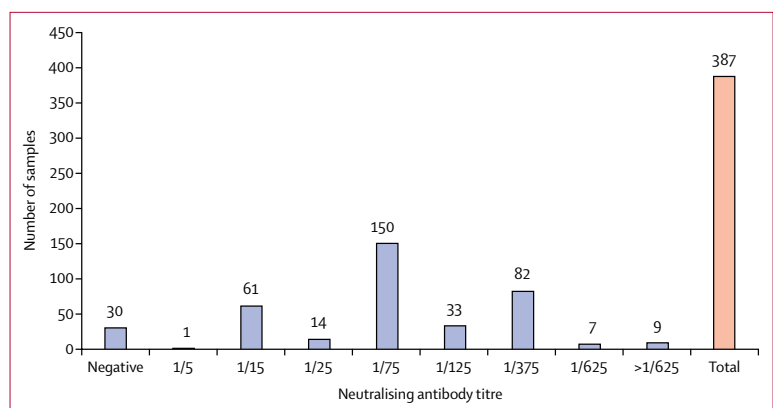


Figure 4: Neutralising antibody titres among the subset of participants included in the immunogenicity analysis

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

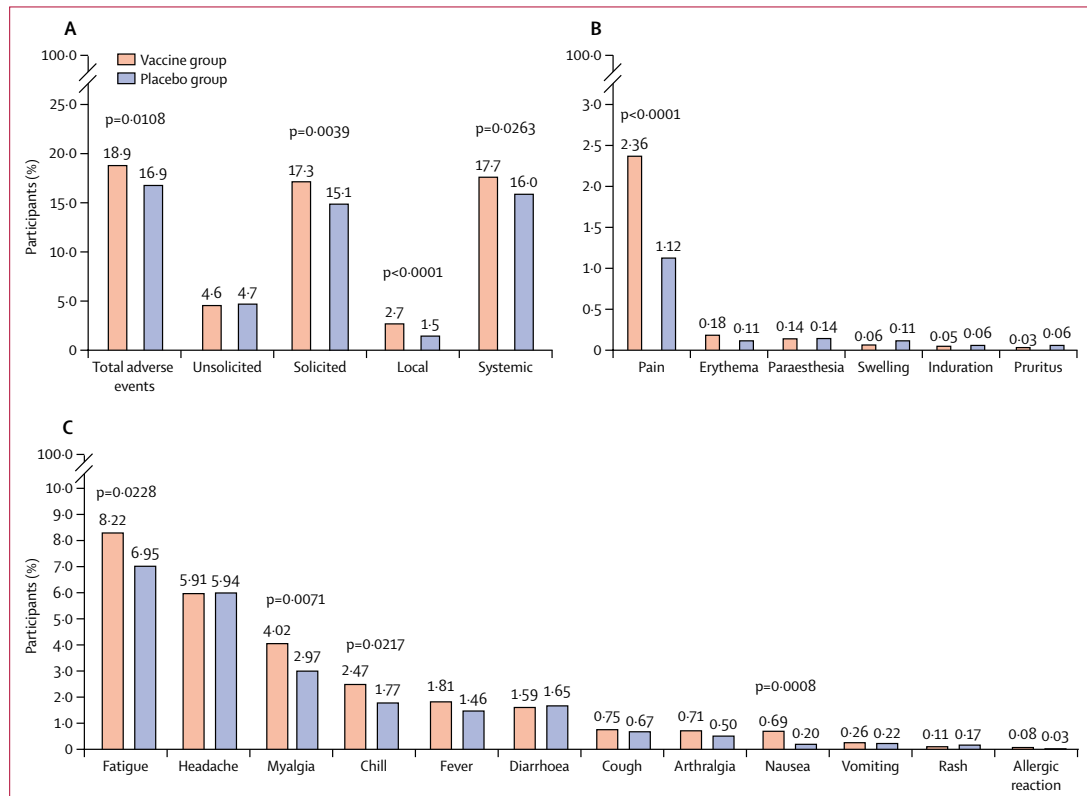


Figure 5: Adverse events (A) Overall adverse events. (B) Local adverse events. (C) Systemic adverse events. p values are shown only for significant differences. See appendix (pp 10–12) for full data.

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 colleagues¹³ 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 colleagues¹⁹ 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 colleagues²² 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.

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1.17. Global efficacy of CoronaVac can reach 62,3% with an interval of 21 days between doses

An article written by Butantan to The Lancet showed that the efficacy of CoronaVac for symptomatic cases reached 50,7% within 14 days of interval between both doses, more than the 50,38% disclosed in January based on the initial data of the phase 3 clinical trials. Besides, the global efficacy, which demonstrates the capacity of the vaccine to protect in mild, moderate and severe cases, may reach 62,3% when the interval between both doses is 21 days or more.

The data is part of a deepening of the clinical studies conducted in 2020 with more than 12.000 participants, all of them healthcare workers. The research was led by the director of clinical trials from Instituto Butantan, Ricardo Palacios. The article also says that the minimum efficacy of the vaccine is already shown in the

second week after the first dose. However, for the immunization to be complete, it is necessary to receive both doses.

Initially, the phase 3 clinical trial showed that, for mild and severe cases that may need medical assistance, the efficacy of the vaccine varied between 78% and 100%. The results of the new research, however, demonstrated that CoronaVac had an efficacy between 83,7% and 100%. It means that CoronaVac can reduce most of the cases that may need some sort of medical care.

The article also suggests that CoronaVac can protect against the variants P.1 and P.2 of SARS-CoV-2.

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- 1 **Article**
- 2 **Title** Efficacy and safety of a COVID-19 inactivated vaccine in healthcare
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25

1 **Abstract**

2 **Background**

3 Vaccines are urgently needed to tackle the unprecedented morbidity and mortality of
4 COVID-19. Administration of inactivated viruses are the common and mature
5 platform of developing new vaccines. CoronaVac is an inactivated vaccine that has
6 undergone preclinical tests and phase I/II clinical trials.

7 **Methods**

8 We conducted a randomised, double-blind, placebo-controlled phase 3 clinical trial
9 with CoronaVac among healthy healthcare professionals in 16 centres in Brazil.
10 Participants received two doses of vaccine (3 µg in 0.5 mL) vaccine or placebo at day
11 0 and 14. The primary efficacy endpoint was the number of symptomatic COVID-19
12 cases confirmed by RT-PCR 14 days after the second dose of the vaccine. Prevention
13 of disease severity was a major secondary efficacy endpoint, and adverse events
14 incidence up to seven days after immunization was the primary safety outcome. The
15 trial was registered at ClinicalTrials.gov, NCT04456595.

16 **Findings**

17 Between July 21 and Dec 16, 2020, 12 396 participants were enrolled and received at
18 least one vaccine or placebo dose. There were 9,823 participants who received the
19 two doses and were followed for at least 14 days and had, therefore, reached the final
20 efficacy analysis. There were 253 confirmed COVID-19 cases in the cohort: 85 cases
21 (11.0/100 person-year) among 4,953 participants in the vaccine group, and 168 cases
22 (22.3/100 person-year) among 4,870 participants in the placebo group. The primary
23 efficacy against symptomatic COVID-19 was 50.7% (95%CI 36.0-62.0). The

1 secondary efficacy against cases requiring assistance (score ≥ 3) and moderate and
2 severe cases (score ≥ 4) were 83.7% (95%CI 58.0-93.7) and 100% (95%CI 56.4-
3 100.0) respectively. All 6 cases of severe COVID-19 occurred in the placebo group.
4 The incidence of adverse reactions, which was mainly pain at the administration site,
5 was higher in the vaccine group (77.1%) than in the placebo group (66.4%). There
6 were 67 serious adverse events reported by 64 participants and all were determined to
7 be unrelated to vaccination, including two fatal cases. In a subset of participants,
8 neutralizing antibody assays showed similar seroconversion and geometric mean titres
9 against B.1.128, P.1, and P.2 variants.

10 **Interpretation**

11 A phase 3 clinical trial conducted in healthcare professionals in Brazil demonstrated
12 that the inactivated CoronaVac vaccine has a good safety profile and is efficacious
13 against any symptomatic SARS-CoV-2 infections and highly protective against
14 moderate and severe COVID-19.

15
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18
19

1 **Introduction**

2 Three coronaviruses (SARS-CoV-1, MERS, and SARS-CoV-2) have been identified
3 as the cause of severe acute respiratory disease in humans this century. An inactivated
4 vaccine was developed for the first of these diseases, SARS, but its development was
5 discontinued in phase I clinical trial because the transmission receded.¹ After the
6 emergence of COVID-19, the same group updated this development using a SARS-
7 CoV-2 strain isolated in January 2020. The new product, later named CoronaVac
8 (Sinovac Life Sciences, Beijing, China), had promising performance in non-clinical
9 studies, as shown by the reduction of disease in non-human primate challenge
10 experiments.² Safety and immunogenicity results in phase I/II clinical trials, in
11 younger³ and older adults⁴, prompted the conduction of this phase III clinical trial.

12
13 Our study focused on healthcare professionals directly caring for or in close contact
14 with COVID-19 patients. The obtention of results in a timely fashion is significant
15 for vaccine development in a pandemic of such proportion and a a major common
16 challenge for all COVID-19 vaccine developers. Brazil has been one of the countries
17 most affected by the COVID-19 pandemic and overall incidence rates have reached
18 high levels, especially in healthcare professionals caring for COVID-19 patients.
19 Therefore, a focus on the latter group was proposed to provide a rapid means to
20 determine the potential efficacy of a vaccine candidate.⁵ This population has been
21 shown to have higher incidence of disease in epidemiological surveys^{6,7} and could, in
22 principle, adhere better to study case surveillance. Therefore, the objective of the
23 present phase III clinical trial was to assess the efficacy and safety of an inactivated
24 COVID-19 vaccine in healthcare professionals. The greater number of presumed

1 cases and a high degree of adherence to the protocol were expected to rapidly meet
2 the research objectives and eventual Emergency Use Authorization for CoronaVac.

4 **Methods**

5 *Study design and participants*

6 This is a phase III multicentre endpoint-driven, randomized, placebo-controlled
7 clinical trial to assess the safety and efficacy of a two-dose schedule of an inactivated
8 COVID-19 vaccine (CoronaVac, Sinovac Life Sciences, Beijing, China) containing
9 aluminium hydroxide adjuvant in healthcare professionals directly dealing with
10 COVID-19 patients. Volunteers were recruited in sixteen clinical sites in Brazil, with
11 1:1 allocation ratio between vaccine and placebo. Initially, the study included only
12 participants aged 18-59 years without previous SARS-CoV-2 infection. After phase
13 I/II data in the elderly population became available,⁴ those with 60 years of age or
14 above were also enrolled, and a study amendment dropped any restriction of prior
15 infection. The primary efficacy objective considered the whole study population
16 regardless of age group and previous infection. The sample size for efficacy was
17 calculated considering an attack rate of 2.5% and one interim analysis. The required
18 number of cases was 61 for the interim analysis and 151 for the primary outcome
19 analysis with estimated recruitment of 13,060 participants. The primary safety
20 objective was incidence of adverse events by age group with up to 11800 participants
21 in the 18-59 years group and up to 1260 in the group of 60 years or older.

22
23 Participants needed to be 18 years of age or older and work as healthcare
24 professionals caring for COVID-19 patients and had to agree to participate by signing
25 the informed consent form. The main exclusion criteria were pregnant or lactating

1 women, unstable chronic disease, previous use of any COVID-19 vaccines, and acute
2 disease symptoms including COVID-19 in the previous 72 hours. The full protocol
3 has been published previously.⁸

4 The study complied with ICH Good Clinical Practices and Brazilian ethical and
5 regulatory guidelines, and was approved by the Brazilian National Research Ethics
6 Council - CONEP - (CAAE 34634620.1.1001.0068) and the Brazilian National
7 Regulatory Agency - ANVISA - (CE 47/2020) and is registered in the
8 ClinicalTrials.gov platform (NCT0445659).

9 10 *Randomization and masking*

11 Two permuted block randomization lists were created according to age group, 18-59
12 years, and 60 years or older. Vaccine and placebo were randomized at a 1:1 ratio and
13 all sites accessed the same randomization lists through an IWRS provided by Cenduit
14 (Durham, NC, USA). Study vaccines and placebos were provided in prefilled syringes
15 with similar characteristics. An unblinded pharmacist at each clinical site prepared the
16 vaccine or placebo. The pharmacist only received a coded request for an experimental
17 product and delivered the randomized product without any contact with the study
18 participant or her/his identification information in a concealed syringe to a blind
19 research staff. Participants and all other study staff as well as monitors, lab
20 technicians, and data management team remained unaware of the product allocation.

21 22 *Procedures*

23 CoronaVac is an inactivated vaccine candidate against COVID-19 derived from the
24 CN02 strain of SARS-CoV-2 grown in African green monkey kidney cells (Vero
25 cells). At the end of the incubation period, the virus was harvested, inactivated with β -

1 propiolactone, concentrated, purified, and finally absorbed by aluminium hydroxide.
2 The placebo was aluminium hydroxide diluent with no virus. Both the vaccine and
3 placebo were prepared in a GMP-accredited facility. Vaccine (3 µg in 0.5ml) and
4 placebo were provided in a ready-to-use syringe and administered intramuscularly
5 following the two-dose schedule of 0,14 (+14) days. The selected vaccine doses have
6 been proven to be sufficient for protection against SARS-CoV-2 challenge in
7 macaques.²

8 This study was carried out in 16 clinical research centres in Brazil. All participants
9 who provided the informed consent were enrolled after baseline assessment of
10 inclusion and exclusion criteria, medical history, physical examination, vital signs,
11 pregnant test, and blood tests. At screening, blood samples and a throat swab were
12 collected for laboratory detection of SARS-CoV-2.

13 CoronaVac or placebo preparation was performed by the unblinded pharmacist at
14 each site and then administered by nurses in a blinded fashion. After vaccination,
15 safety evaluation was conducted by investigators who were unaware of treatment
16 assignments onsite for 60 minutes. Follow-up contacts were allocated to each
17 participant to verify the occurrence of adverse events and COVID-19 symptoms.
18 These contacts could be made electronically, by telephone, or in-person, at the
19 discretion of the study team and the participant informed the team about the means of
20 contact they preferred. Contacts were made between the third and fifth day after each
21 vaccination and thereafter every week for the first 13 weeks after vaccination and
22 every two weeks for the remainder of the study. Once fever or other symptoms related
23 to COVID-19 was reported, the participants were asked to seek assistance from the
24 study team to collect a throat swab to diagnose COVID-19. All possible cases were

1 followed up to the resolution of all symptoms and the duration and severity of each of
2 the signs and symptoms documented.

3 An independent data and safety monitoring committee was established prior to the
4 study initiation. Safety data were assessed and reviewed by the committee to ensure
5 safety.

6

7 *Outcomes*

8 The primary endpoint was the efficacy of CoronaVac against confirmed symptomatic
9 COVID-19 with onset at least 14 days after the second injection in the *per protocol*
10 population. All the cases were judged by a blind independent clinical endpoint
11 adjudication committee. Confirmed COVID-19 cases were defined as: 1) at least two
12 consecutive days with one or more specific symptoms (cough, newly developed taste
13 or smell disorders, shortness of breath or dyspnea); or 2) with two or more non-
14 specific symptoms (fever [axillary temperature $\geq 37.5^{\circ}\text{C}$], chills, sore throat, fatigue,
15 nasal congestion or runny nose, body pain, muscle pain, headache, nausea or
16 vomiting, diarrhoea; or 3) imaging features of COVID-19; and 4) detection of SARS-
17 CoV-2 nucleic acid in respiratory swab by RT-PCR. A case definition based on the
18 U.S. Food and Drug Administration (FDA) criteria was also used as a sensitivity
19 analysis.⁹ Following the latter criteria, a positive case was considered as anyone who
20 presented at least one of the following symptoms for two days or more, with a
21 positive SARS-CoV-2 RT-PCR result: fever or chills, cough, shortness of breath or
22 difficulty in breathing, fatigue, muscle or body pain, headache, sore throat, nasal
23 congestion or runny nose, nausea or vomiting, and diarrhoea. The primary efficacy
24 was also evaluated in distinct subgroups, including age groups, race, and ethnic group,
25 with or without underlying medical conditions, different vaccination intervals

1 between two doses (<21 days or ≥ 21 days), and severity of COVID-19 according to
2 WHO Clinical Progression Scale.¹⁰ A modified intention-to treat analysis was also
3 performed to verify the exploratory aim of evaluating the efficacy after a single dose.
4 All the cases included for efficacy analysis had symptoms initiating up to December
5 16, 2020.

6 The primary safety endpoint was incidence of adverse reactions within 7 days after
7 injection. The safety profile was assessed based on the safety set (SS), consisting of
8 all the participants who received at least one dose vaccination. The events included in
9 this analysis were those initiating up to December 16, 2020 and corresponded to a
10 median follow-up of two months after the second dose.

11 Serum samples from a subset of the first participants per age group of the
12 coordinating clinical site were analysed to determine neutralization titres by
13 cytopathic effect-based virus neutralization test (CPE - VNT) using SARS-CoV-2
14 wild-type variants: B.1.128 (SARS-CoV-2 / human / BRA / SP02 / 2020 strain
15 (MT126808.1), SARS-CoV-2-P.1 (MAN 87201 strain) and SARS-CoV-2-P.2 (LMM
16 38019 strain) in 96-well plates containing $5E+04$ cells / mL of Vero cells (ATCC
17 CCL-81). All procedures related to VNT were performed in a level 3 biosafety
18 laboratory, from the Institute of Biomedical Sciences of the University of São Paulo,
19 following WHO recommendations.

20

21 *Statistical analysis*

22 The primary efficacy analysis of was a -modified *per protocol* analysis calculated
23 with all virologically confirmed cases of COVID-19 occurring in the period from the
24 beginning of vaccination to two weeks after the second dose, using Cox proportional
25 hazards regression model. This model calculates the estimated vaccine efficacy (1 -

1 hazard ratio), and the Wald test based on the Cox model compared to the p-values
2 described above, and 95% confidence interval according to the appropriate alpha level
3 was similarly transformed and presented. Cumulative incidence charts were also
4 created with this model. The hypothesis test of the primary efficacy endpoint in the
5 *per protocol* population was based on the on each analysis' alpha spent levels and
6 followed up with the corresponding confidence intervals. Interim efficacy analysis
7 was set to be triggered upon collection of at least 61 primary endpoint cases. The
8 safety analysis included all participants who received at least one dose of CoronaVac
9 or placebo. For neutralization assays, seroconversion was defined as a person with a
10 post-vaccination titre ≥ 20 with a baseline negative result. The Geometric Mean Titres
11 (GMT) were also calculated for those that seroconverted in each group. The Pearson
12 Chi-square test or Fisher's exact test was adopted for the analysis of categorical
13 outcomes. The 95% confidence intervals (95% CIs) of categorical outcomes were
14 computed with the Clopper-Pearson method. Hypothesis testing was two-sided and P-
15 values < 0.05 was considered statistically significant.

16

17 *Role of the funding sources*

18 Employees of Fundação Butantan and Instituto Butantan participated in the study
19 design, data collection, data analysis, data interpretation, and the report writing. Those
20 organizations are non-profit. All the authors have full access to all the data in the
21 study and the corresponding authors had final responsibility for the decision to submit
22 for publication.

23

1 Results

2 From July 21 to December 16, 2020, 12,842 participants were screened, and 12,408
3 were randomized at 16 study sites in Brazil. A total of 12,396 participants received at
4 least one dose of CoronaVac or placebo (Figure 1), 6,195 in the vaccine group and
5 6,201 in the placebo group.

6 Among those 12,396 participants, 5.1% were elderly participants aged 60 years or
7 older, 64.2% were female, and most participants self-identified themselves as white
8 (75.3%). More than half of the participants (55.9%) had underlying diseases, 22.5%
9 of them were obese ($\text{BMI} \geq 30 \text{ kg/m}^2$). The average age and BMI of participants were
10 39.5 years and 26.8 kg/m^2 , respectively (Table 1).

11 All 12,396 participants were involved in the safety set (SS) and monitored for adverse
12 events from the beginning of vaccination up until 12 months after the first dose
13 vaccination. By the cut-off date, the incidence of adverse events and adverse reactions
14 were 78.8% and 71.7%, respectively, by the cut-off date (Appendix p6). Generally,
15 the vaccine group reported more adverse reactions than the placebo group (77.1% vs.
16 66.4%; $p < 0.0001$), and most adverse reactions were solicited (73.1% vs. 60.0%,
17 $p < 0.0001$) (Figure 2A).

18 Among solicited adverse reactions, the incidence of local adverse reactions was
19 61.5% in the vaccine group, and this was higher than the 34.6% in the placebo group
20 ($p < 0.0001$). Local adverse reactions were mainly driven by pain at the injection site
21 (60.3% vs. 32.5%, $p < 0.0001$). All solicited local reactions were more frequently in
22 the vaccine group, and the incidences were less than 6% in the vaccine group, except
23 pain at the injection site (Figure 2B). Systemic adverse reactions were similar in the
24 vaccine and placebo groups (48.4% vs. 47.6%, $p = 0.3882$), including headache and

1 fatigue, the most common systemic symptom collected in this trial. Myalgia was more
 2 frequent in the vaccine group (11.7% vs. 10.5%, $p=0.0257$). Fever ($\geq 37.8^{\circ}\text{C}$) was
 3 rare and only reported by 0.2% and 0.1% ($p=0.2666$) participants in the vaccine and
 4 placebo groups, respectively (Figure 2C). Unsolicited ARs were reported by 36.8% in
 5 the vaccine and 35.8% in the placebo groups ($p=0.2177$, Figure 2A). Only tremor,
 6 flushing and local reactions in the administration site (reported in an unsolicited
 7 period) showed higher incidence in the vaccine group. No difference was found for
 8 other unsolicited symptoms (Appendix p7-10).

9 In this study, 67 serious adverse events were reported by 64 participants, 33 in the
 10 vaccine group and 31 in the placebo group (Appendix p20-23). The overall incidence
 11 of SAE was 0.5%. All SAEs were determined as unrelated to the vaccine. Two deaths
 12 were reported in this trial: one case of cardiopulmonary arrest (placebo group), and
 13 one case of medication overdose (vaccine group); all of them unrelated to the vaccine.
 14 One additional death due to COVID-19 (placebo group) occurred as outcome on an
 15 ongoing case by the data cut time.

16 Among 9,823 participants in the *per protocol* analysis, 253 cases of symptomatic
 17 COVID-19 were reported during the primary efficacy analysis period (Table 2). There
 18 were 85 cases (11.0/100 person-year) among 4,953 participants in the vaccine group,
 19 and 168 cases (22.3/100 person-year) among 4,870 participants in the placebo group.
 20 The efficacy to prevent symptomatic COVID-19 was 50.7% (95%CI 35.9-62.0).
 21 Considering the α spending in the interim analysis, the corrected efficacy was 50.7%
 22 (95.4%CI 35.7-62.2). Sensitivity analysis of primary efficacy was conducted based
 23 on other case definitions, and the efficacy results ranged from 51.2% to 54.1%
 24 (Appendix p24).

1 A key secondary endpoint was to evaluate the efficacy to prevent COVID-19 disease
2 at different clinical severities. There were 35 cases scored 3 and above, 10 cases
3 scored 4 and above, 6 severe cases (including one fatal case) reported among the 9823
4 participants. For cases scored 3 and above, 5 cases were in the vaccine group, 30 were
5 in the placebo group, resulting in a vaccine efficacy of 83.7% (95%CI 58.0-93.7). All
6 cases scored 4 and above were in the placebo group, resulting in 100% vaccine
7 efficacy against moderate and severe cases (95%CI 56.4-100.0).

8 Subgroup analyses were also conducted by the interval between two doses, the
9 exposure status to SARS-CoV-2 pre-vaccination, age group, and underlying disease.
10 Participants with two doses interval of fewer than 21 days showed similar efficacy
11 (49.1%; 95%CI 33.0-61.4) as the primary efficacy analysis. For the small portion of
12 participants who received two doses of vaccine or placebo with an interval of 21 days
13 or more, the efficacy was calculated at 62.3% (95%CI 13.9-83.5). The efficacy was
14 similar between different exposure status to SARS-CoV-2 pre-vaccination
15 (Unexposed: 50.5%; Exposed: 49.5%), and between other age groups (18 to 59 years:
16 50.7%; ≥ 60 years: 51.1%). For participants with underlying diseases, a total of 130
17 cases were reported in this population, resulting in 48.9% efficacy (95%CI 26.6-
18 64.5). For participants with cardiovascular disease, diabetes, and obesity, the efficacy
19 was 39.5% (95%CI -66.4-78.0), 48.6% (95%CI -115.3-87.7) and 74.9% (95%CI
20 53.7-86.4), respectively. Two-hundred and fifty participants of Asian ethnicity
21 reported 4 cases, of which 1 in the vaccine group and 3 in the placebo group, resulted
22 in 66.0% efficacy (95%CI -226.8-96.5).

23 After the first dose or 14 days after the first dose, secondary efficacy endpoints were
24 analysed using the intention-to-treat (ITT) approach. Among the 12,396 participants,

1 378 cases were reported after the first dose, of which 126 were in the vaccine group
 2 and 252 were in the placebo group, resulting in an efficacy of 50.8% (95%CI 39.0-
 3 60.3) after the first dose, similar to the calculated efficacy with the complete
 4 vaccination schedule. For 14 days after the first dose, 313 cases were collected among
 5 11,431 participants, 94 were in the vaccine group and 219 were in the placebo group,
 6 resulting in an efficacy of 57.9% (95%CI 46.4-66.9) (Figure 3).

7 One hundred and nine participants had samples processed for neutralization assay
 8 before vaccination and two weeks after the second dose. Six of them had positive pre-
 9 vaccination samples (four for the vaccine and two for the placebo groups) and were
 10 not included in the seroconversion assessment. Two of four vaccinated participants
 11 with previous antibody titres had a 4-fold increase or higher for all tested variants.
 12 Three participants (5.2%) out of 58 in the placebo arm seroconverted for the variant
 13 B.1.1.28, but not to the other variants. Thirty-two (71.1%; GMT 64.4) of the 45
 14 participants vaccine arm seroconverted for B.1.1.28, 31 (68.9%; GMT 46.8) for P.1,
 15 and 36 (80.0% GMT 45.8) for P.2. There were no significant differences in GMT
 16 against the B.1.128 variant as compared to P.1 GMT ($p=0.34$) and P.2 GMT ($p=0.72$).

17 In vaccinated individuals who seroconverted, 21 of 22 (95.5%; GMT 72.8) adults
 18 aged 18 to 59 years, 21 had seroconversion for B.1.1.28, 17 of 22 (77.3%; GMT 60.9)
 19 for P.1 and 21 of 22 (95.5%; GMT 50.4) for P.2. Of the 23 samples analysed from
 20 participants aged 60 years or more, 11 (47.8%; GMT 58.1) evidenced seroconversion
 21 for B.1.1.28, 14 (60.9%; GMT 34.5) for P.1, and 15 (65.2%; GMT 40.0) for P.2.
 22 When the different age groups are compared, there were significant in seroconversion
 23 rates for B.1.1.28 ($p<0.001$) and P.2 ($p=0,022$) variants, but not for the P.1 variant
 24 ($p=0.337$). The differences in GMT between age groups were not significantly

1 different for the B.1.1.28 variant ($p=0.086$) nor the P.2 variant ($p=0.174$) but was
2 different for the P.1. variant ($p=0.029$).

3

4 **Discussion**

5 The PROFISCOV study was designed to test CoronaVac in a group exposed to
6 SARS-CoV-2 more often and at potentially higher infectious doses than in a
7 community exposure. Using a smaller sample size compared to other large Phase III
8 clinical trials with vaccine candidates, we were able to demonstrate that this vaccine
9 was safe, well-tolerated, and efficacious. Efficacy to prevent any symptomatic
10 COVID-19 started at 50.7% and became more extensive as disease severity increased.
11 Of note, the case definition and professional profile of the study population allowed
12 highly sensitive surveillance and the study was able to detect even the mildest cases of
13 COVID-19. The conditions of this trial should be considered when the results are
14 extrapolated to other populations or comparisons with other trials are suggested.
15 The vaccine performance met the requirements for Emergency Use Authorization in
16 32 countries and regions allowing a fast response to an ongoing public health
17 emergency at a speed similar to other vaccine candidates receiving heavy subsidies
18 from governments and international organizations.
19 One of the factors that might have affected the study's overall efficacy was the
20 interval between two doses of 14 days. Although there were a limited number of
21 participants in this study having doses with an interval of 21 days or higher, there was
22 a trend to higher efficacy. Furthermore, previous neutralization data in adults were
23 lower with a 14-days interval³, and, in this study, participants aged 60 years or more
24 had a lower response than adults with the same 14-days schedule. These results
25 contrast with previous studies where the immune responses in adults and elderly

1 populations with a 28-days interval schedule were comparable^{3,4}. Taken together,
2 these data suggests that it is advisable to encourage longer intervals between doses,
3 i.e., 28 days, in the vaccine implementation. The study cannot make a clear
4 assumption of efficacy with a single dose due to the limited number of outcomes and
5 the odds of having more participants infected around the time of first injection in the
6 vaccine arm (Figure 3). However, it must be noticed that the efficacy of CoronaVac
7 was already present after the second week of the first dose.

8 The study was not designed to provide subgroup efficacy analysis by previous SARS-
9 CoV-2 exposure, age group, or underlying medical conditions. Nonetheless, the
10 efficacy found in participants with obesity is promising because this condition has
11 been associated with lower immune response in other inactivated vaccines.¹¹

12 There is international concern that the emergency of SARS-CoV-2 variants may alter
13 vaccine efficacy. Two variants have emerged in Brazil after this trial started, the so-
14 called P.2 and P.1. Out of them, only the P.2 variant was circulating on the study
15 centres during the period covered by this analysis. Although these variants have
16 several mutations that are key to the function of many antibodies, there was a
17 consistent neutralization of all these variants by serum of participants given the
18 inactivated vaccine. This is expected as the vaccine contains the whole virus.

19 The observed safety and tolerability profiles were outstanding. As it was observed
20 with other COVID-19 vaccines, no vaccine-enhanced disease effect was documented,
21 besides post-implementation surveillance is advisable.¹² Local pain was the most
22 frequent adverse reaction. Differences in adverse event rates between experimental
23 and control products became an issue in several COVID-19 vaccine developments, as
24 study blinding could be compromised leading to changes in participant behaviour.

1 Since CoronaVac showed similar reactogenicity to placebo, such concern was not an
2 issue in this trial.

3 This pivotal trial for CoronaVac was able to demonstrate the safety and efficacy of a
4 new COVID-19 vaccine with one of the most efficient approaches among first-wave
5 developers maintaining the highest standards in science and ethics. After the results of
6 this study were initially released on January 12, 2021, Butantan have delivered 38,2
7 million doses to the Brazilian Public Health System and Sinovac distributed
8 additional 180 million doses in around 30 low-and-middle-income countries up to
9 April 07, 2021. The deployment rate of this vaccine was higher and more opportune
10 for those countries than other initiatives ¹³ demonstrating the success of the Sinovac-
11 Butantan co-development and confirming that the use of traditional inactivated virus
12 vaccine strategies cannot be ruled out as a platform of rapid public health response to
13 epidemics or pandemics caused by emerging pathogens, such as SARS-CoV-2.

14
15

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22

23

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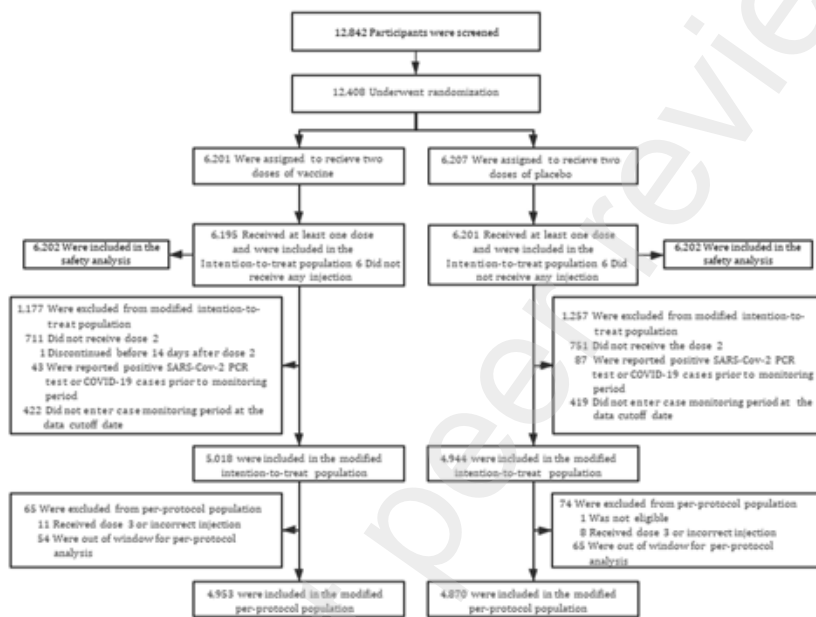
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21

1 **Figure legends**

2 **Figure 1: Study Profile.**

3 All participants enrolled from Jul. 21 to Dec. 16, 2020, were shown in the diagram.

4



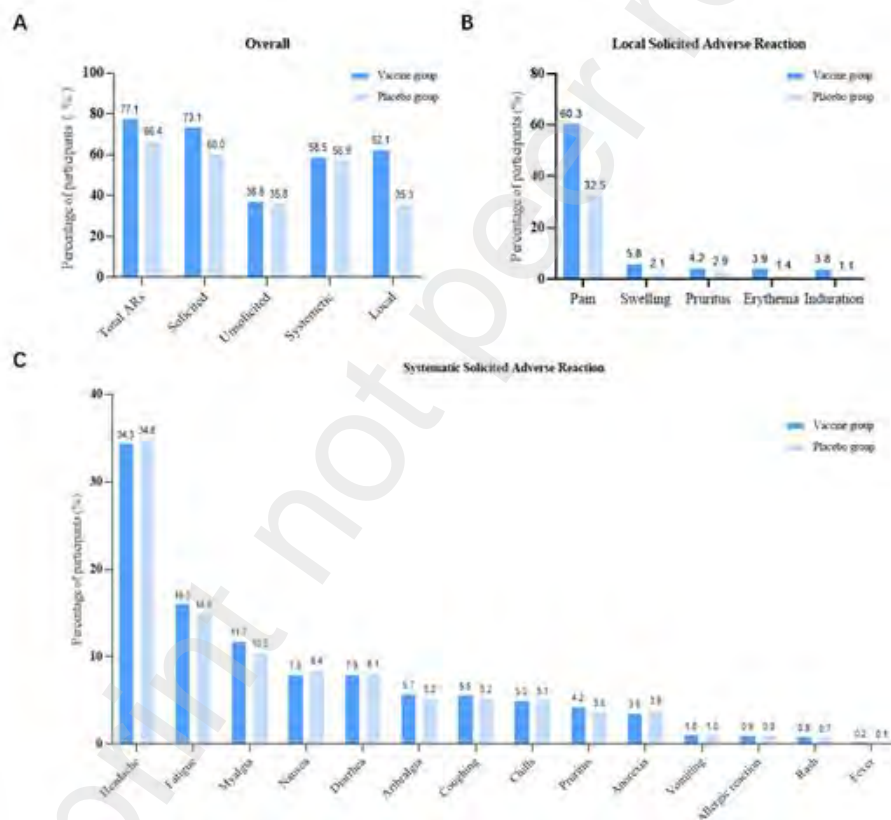
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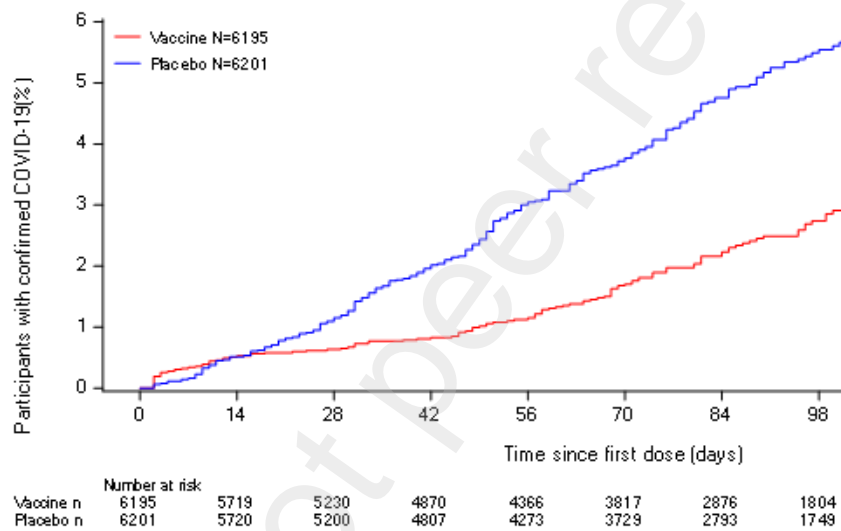
1 **Figure 2: Overview of Adverse Reactions and Solicited Local/Systemic Adverse**
 2 **Reactions.**

3 The percentage of participants who had adverse reactions after any administration of
 4 vaccine or placebo was shown. (A) The overview of the percentage of participants who
 5 had any adverse reactions; (B) The percentage of participants who had local solicited
 6 adverse reactions by different symptoms; (C) The percentage of participants who had
 7 systematic solicited adverse reactions by different symptoms.
 8



9
 10
 11

1 **Figure 3. Efficacy of vaccine against COVID-19 cases after the 1st dose and the**
 2 **Kaplan-Meier cumulative incidence curves**
 3 (A) The Kaplan-Meier cumulative incidence curves of symptomatic Covid-19 cases
 4 after the 1st dose of vaccination. (B) The number of cases collected, incidence density,
 5 and efficacy of 14 days after the 1st dose and 2nd dose. Analysis was based on the
 6 intention-to-treat population; Incidence density: per 100 person-years.
 7 A



8
 9
 10 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 1 st dose	313	94/5717(8.0)	219/5714(19.0)	57.9 (46.4, 66.9)
14 days after 2 nd dose	253	85/4953(11.0)	168/4870(22.3)	50.7 (35.9, 62.0)

11

1 **Tables**

2 **Table 1: Baseline characteristics of participants who received at least one dose of**
 3 **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)

1 Data are n (%) and mean (SD).

2

3

1 **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	Vaccine	Placebo	Vaccine Efficacy
	No. of			(95% CI)
	cases	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 ^[1] The efficacy corrected based on the α spending in the interim analysis was 50.7%
- 2 (95.4% CI: 35.7, 62.2).
- 3 ^[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.

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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	Gecilmar 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
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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

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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 neuritis	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)

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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

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Sinovac Biotech Co., Ltd, Beijing, People's Republic of China

Gang Zeng, Qianqian Xin, Weining Meng

1.18. Butantan's vaccine has global efficacy superior to the one demanded by WHO

The Instituto Butantan and the Government of São Paulo said that CoronaVac had 50,38% of global efficacy in the clinical trial developed in Brazil, and the protection of 78% in mild cases and 100% in moderate and severe cases of Covid-19. All the indexes are superior to the baseline of 50% demanded by WHO (World Health Organization).

The results were submitted to an independent international committee and are already with Anvisa (National Health Surveillance Agency), that is analyzing the request of emergency use of the vaccine in Brazil. The research involved 16 scientific research centers in 7 states and in the Federal District. The double blinded test had 12,5 thousand healthcare workers as volunteers.

“It is an excellent vaccine waiting to be used in a country where around one thousand people are dying each day. We hope the authorities understand the urgency of the moment and help our population receive the vaccines as fast

as possible”, said the President of Instituto Butantan, Dimas Covas.

“These results are extremely important for public health, and can prevent people from getting sick and overloading the hospitals. It's our chance to avoid people from dying”, said the State Secretary of Health, Jean Gorinchteyn. “We have a vaccine that was tested in real life, in the middle of a pandemic and on those that were the most exposed”, concluded him.

The study verified that from a sample of 9,2 thousand participants, 85 of the very mild cases were from people that received the vaccine, and 167 of volunteers that received placebo.

The results of efficacy in the moderate cases, classified as score 3, in patients that needed to receive some sort of assistance, was 77,96%. Only seven vaccinated people had the moderate disease, against 31 of the placebo group.

For the severe cases that required

hospitalization, the efficacy was 100%. None of the infected patients that received the vaccine from Butantan needed hospitalization. Among those that received placebo, there were seven patients that needed to be hospitalized.

All the volunteers are healthcare workers, with a high and continuous risk of being exposed to the coronavirus. They received two doses of the vaccine, with a gap of two weeks between the application. The research also demonstrated that the vaccine is extremely safe - no severe adverse reactions were reported by the participants.

The vaccine is developed by Butantan in an international partnership with the biopharmaceutical Sinovac Biotech, in Beijing, China. The product is based on the inactivation of SARS-CoV-2 to induce the immunological human system to

react against the virus. The technology is similar to other vaccines widely produced by the institute in São Paulo.

In November 2020, the Lancet journal, one of the most relevant in the world, published the results of safety of the vaccine from Butantan in phases 1 and 2, conducted in China, with 744 volunteers. The article showed that the product is safe and capable of inducing immune response in 97% of the cases until 28 days after the application.

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**SÃO PAULO**
GOVERNO DO ESTADO

**Vacina do Butantan
A vacina do Brasil**

2021

**A VACINA DO BUTANTAN
É SEGURA**

 **INSTITUTO
BUTANTAN**
A serviço da vida

**SÃO PAULO**
GOVERNO DO ESTADO

O PRINCIPAL PAPEL DE UMA VACINA É SALVAR VIDAS



OS GRUPOS DE MAIOR RISCO SÃO SEMPRE PRIORIDADES



E PARTINDO DESSE PRINCÍPIO,
O **INSTITUTO BUTANTAN**
APRESENTA SEU
ESTUDO DE EFICÁCIA DA
VACINA DO BUTANTAN



É O ESTUDO QUE INCLUIU **MAIS**
VOLUNTÁRIOS NO BRASIL



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



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**
- **Deteção precoce** de casos
- **Dois doses** com intervalo de duas semanas



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

Escala de Progressão da OMS de COVID-19



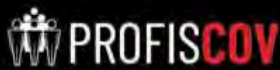
Escala de Progressão em relação à eficácia



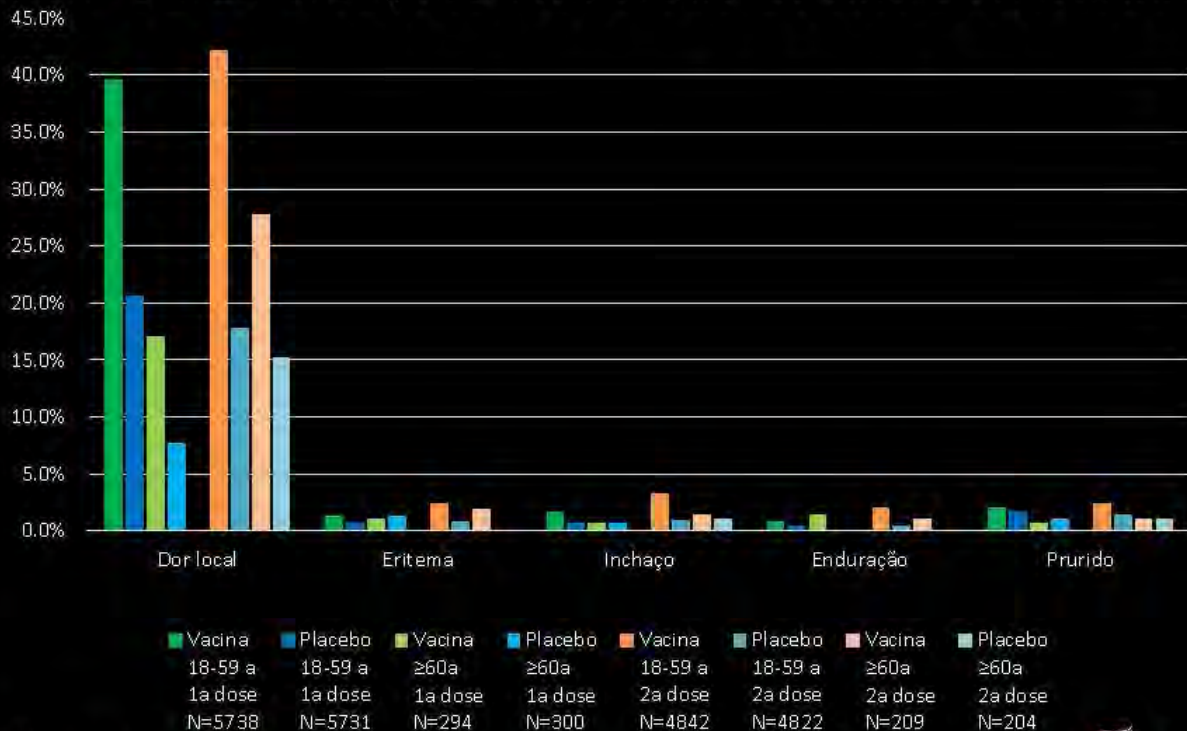
Efeito vacinal conforme a escala de progressão



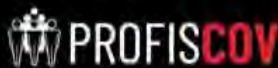
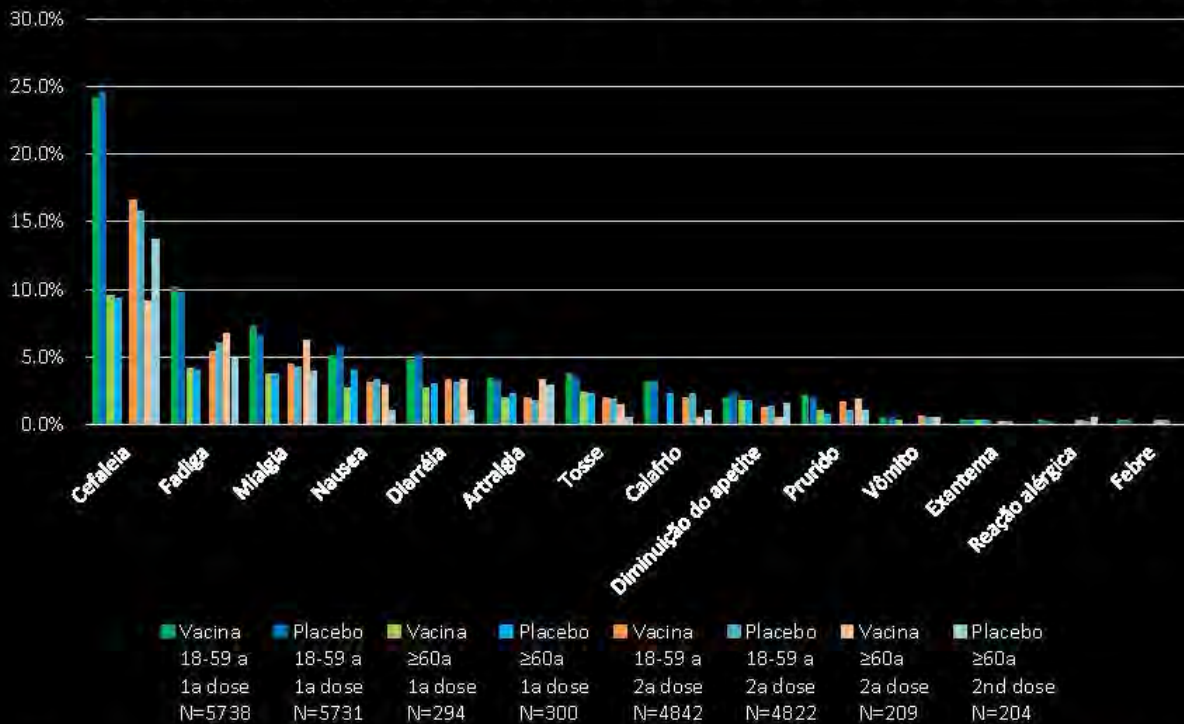
Avaliação conforme a escala de progressão



COV-02-IB Reações Adversas Locais Solicitadas



COV-02-IB Reações Adversas Sistêmicas Solicitadas

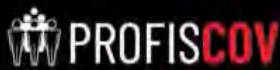
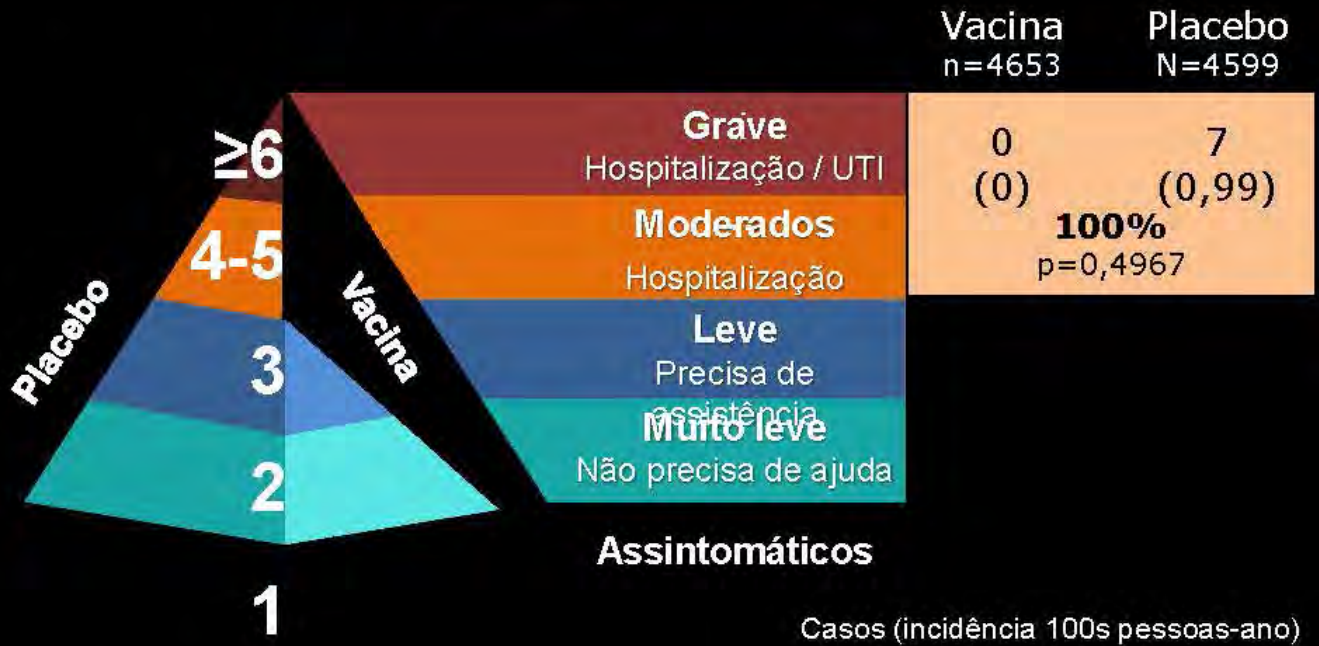


Conclusões de segurança

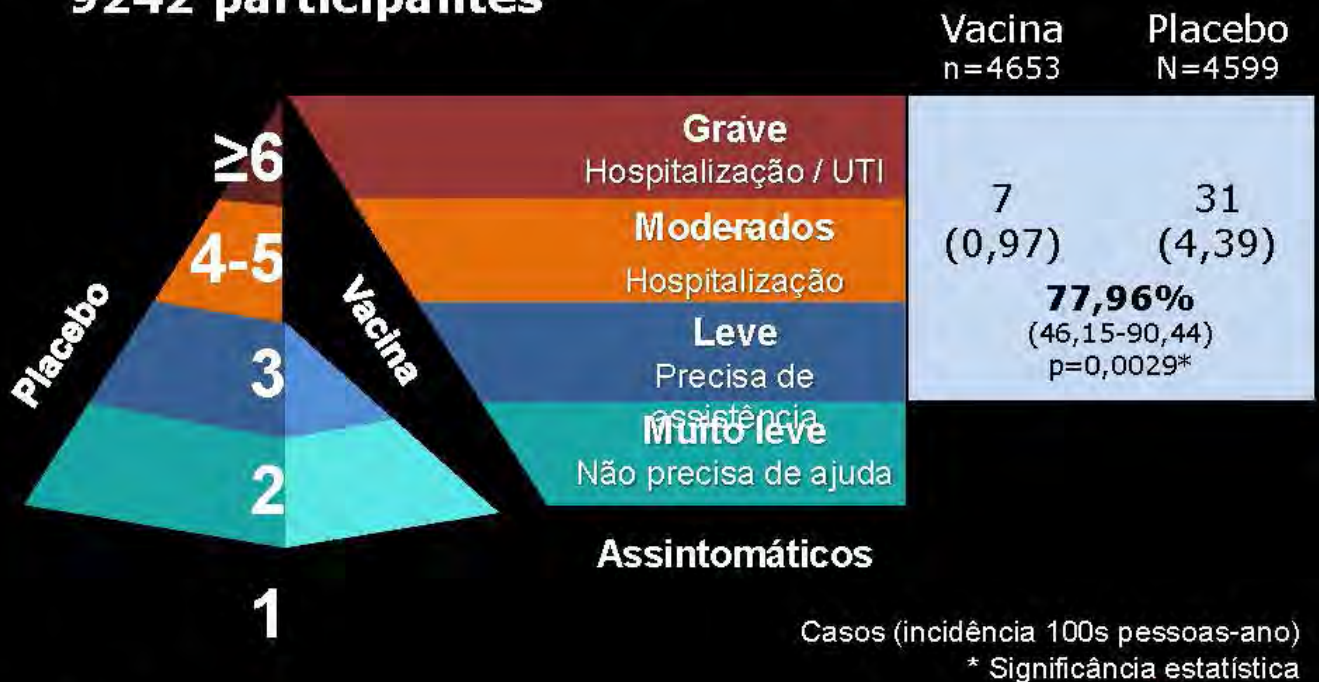
- **Não foram registrados** eventos adversos graves e de interesse especial relacionados à vacinação
- **Reações alérgicas ocorreram em 0,3% dos participantes, não foi observada reação anafilática e sem diferenças entre o grupo experimental e placebo**



Resultados de eficácia - Score 4 ou superior 9242 participantes



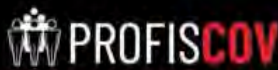
Resultados de eficácia - Score 3 ou superior 9242 participantes



Resultados de eficácia - Score 2 ou superior 9242 participantes



Casos (incidência 100s pessoas-ano)
* Significância estatística



Resultados de eficácia - Score 2 ou superior 9242 participantes



Casos (incidência 100s pessoas-ano)
* Significância estatística



Resumo de resultados de eficácia 9242 participantes



* Significância estatística



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**



Estratégias

Vírus atenuado, morto ou VLP



Proteínas virais



Vetores virais



RNAm e DNA



<https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html>



Desenvolvimento

		U. Oxford / Astra Zeneca
		CanSino / Beijing Inst Tech
		Gamaleya
		Jansen / Johnson & Johnson
		Sinovac
		Wuhan Inst Biol / Sinopharm
		Beijing Inst Biol / Sinopharm
		Barath
		Moderna / NIAID
		BioNTech / Pfizer
		Curevac
Novavax		
AnGes / Osaka University / Takara Bio		
Bektop		
Anhui Zhifei Longcom		
Medicago / GSK		
Clover / GSK / Dynavax		
Murdoch Children's Research Institute		



<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>



Eficácia dos estudos

- Pfizer **95%**
- Moderna **94,1%**
- Gamaleya **90%**
- Sinopharm **79%**
- AstraZeneca **62 a 90%**

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

Clinical trials of CoronaVac around the World



Turkey : Phase III ,Sep.16th
13000 Health Care Workers + General
Population, 18-59 years

China : Phase I/II ,Apr.16th
744 General Population,18-59 years
422 General Population, ≥ 59 years



Brazil : Phase III , Jul.21st
13060 Healthcare Workers,
≥18 years



Indonesia : Phase III ,Aug.11st
1620 General Population, 18-59 years

Chile : Phase III ,Nov.27th
3000 Health Care Workers + General
Population, ≥18 years



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

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

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



Obrigado

1.19. Studies confirm the safety of the coronavirus vaccine developed in partnership with Butantan

CoronaVac vaccine, developed in partnership with Instituto Butantan, proved to be safe and with a good immunogenicity index. The finding is from a study published by the Chinese pharmaceutical company Sinovac Life Science. The research analyzed the behavior of 600 volunteers vaccinated in China during phase 2 of clinical trials.

Each volunteer received two doses, with half of the participants taking the vaccine itself and the other half taking a placebo. According to the results of the studies, there is no concern about the safety of the vaccine used in the volunteers. Among the main adverse reactions is the mild pain at the application site.

The vaccine developed by Sinovac Life Science is one of the most promising in the world because it uses technology already known and widely applied in other vaccines. Instituto Butantan estimates that its incorporation into the health system should occur more easily.

The Asian laboratory has already conducted tests in about a thousand volunteers in China, in phases 1 and 2. Previously, the experimental model applied in monkeys showed expressive results in terms of immune response against SARS-CoV-2.

The pharmaceutical company provided Butantan with the vaccine doses to conduct phase 3 clinical trials in volunteers in Brazil, aiming to demonstrate its efficacy and safety.

If the vaccine is approved, technology will be transferred for scale-up production and free delivery by SUS. The next steps will be the registration of the immunizer by Anvisa (National Health Surveillance Agency) and distribution throughout Brazil.

Published on : 08/10/2020

1 **Full title**

2 **Immunogenicity and Safety of a SARS-CoV-2 Inactivated Vaccine in Healthy**
3 **Adults Aged 18-59 years: Report of the Randomized, Double-blind, and**
4 **Placebo-controlled Phase 2 Clinical Trial**

5 **Running title**

6 Phase 2 Clinical Trial of SARS-CoV-2 Inactivated Vaccine

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69 **Footnote**

70 *The first six authors, YZ, GZ, HP, CL, BK, and YH, contribute equally to the
71 manuscript and are listed as the first authors.

72 ¶The last five authors, JL, XW, MX, QG, and FZ, contribute equally to the
73 correspondence and are listed as the corresponding authors.

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77 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.

105 BACKGROUND

106 In January 2020, outbreaks of coronavirus disease in 2019 (COVID-19) caused by
107 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) escalated rapidly,
108 and since then COVID-19 cases have been reported in over 200 countries and
109 territories. The pandemic continues to spread unabated affecting the health and
110 changing the lifestyles of people globally.¹ To reduce the disease burden and stop the
111 community-wide transmission of COVID-19 across the globe, specific therapeutic
112 agents or vaccines are urgently needed. Till now, more than 120 vaccine candidates
113 have been reported to be under development and at least 23 have progressed to the
114 clinical evaluation stage.²

115 The inactivated SARS-CoV-2 vaccine with aluminum hydroxide developed by
116 Sinovac Life Sciences Co., Ltd., also known as CoronaVac, has been shown to be safe
117 and could induce SARS-CoV-2 specific neutralizing antibodies in mice, rats, and
118 nonhuman primates.³ On the basis of the results obtained from our phase 1 trial, no
119 safety concerns have been identified. Notably, immunization of CoronaVac induced
120 immune responses against SARS-CoV-2 in adults. Here, we report the results of the
121 phase 2 trial.

122 METHODS

123 TRIAL DESIGN AND OVERSIGHT

124 This double-blind, randomized and placebo-controlled phase 2 clinical trial based on a
125 seamless design was registered at clinicaltrials.gov (NCT04352608) and was
126 conducted in Suining County, Jiangsu Province, China. Detailed information about
127 the trial has been provided in our previous phase 1 study. The trial protocol and the

128 informed-consent form were approved by the ethics committee of the Jiangsu
129 Provincial Center for Disease Control and Prevention (JSCDC). This clinical trial was
130 conducted in accordance with the Chinese regulatory requirements and the standards
131 of good clinical practice.

132 Before enrollment, written informed consent was obtained from each participant. The
133 main exclusion criteria included high-risk epidemiological history, positive IgG, IgM
134 or nucleic acid test of pharyngeal or anal swab, axillary temperature $>37.0^{\circ}\text{C}$, allergy
135 to a vaccine component, and other unsuitable conditions.

136 A total of 600 healthy adults aged 18-59 years were randomly assigned into 3 groups
137 in a ratio of 2:2:1 to receive 2 injections of the trial vaccine at a dose of $3\ \mu\text{g}/0.5\ \text{mL}$
138 or $6\ \mu\text{g}/0.5\ \text{mL}$, or placebo on a Day 0,14 schedule or a Day 0,28 schedule, according
139 to a random list generated by an independent statistician..

140 VACCINE

141 The vaccine candidate was an inactivated SARS-CoV-2 whole virion vaccine with
142 aluminium hydroxide as adjuvant (CoronaVac) developed by Sinovac Life Sciences
143 Co., Ltd. SARS-CoV-2 virus was propagated in Vero cells and harvested. The
144 harvested virus was inactivated using β -propiolactone and further purified. The bulk
145 vaccine material obtained from this step was then adsorbed onto aluminium hydroxide
146 and formulated with phosphate-buffered saline (PBS) and sodium chloride as
147 inactivated final product. The dosage of $3\ \mu\text{g}/0.5\ \text{mL}$ and $6\ \mu\text{g}/0.5\ \text{mL}$ were adopted in
148 this study. Whereas the placebo contained aluminum hydroxide diluents with no
149 antigen. Both were administered intramuscularly on the schedule of Day 0,14 or Day
150 0,28.

151 SAFETY ASSESSMENT

152 For safety evaluation of CoronaVac, the participants who received at least one dose of
153 vaccination was included. All vaccinated subjects were observed for immediate
154 adverse events (AEs) on-site for at least 30 minutes after each administration. Diary
155 cards were issued to the participants to record the solicited AEs (e.g. pain, induration,
156 swelling, redness, rash, pruritus) occurring on day 0~7 and unsolicited AEs (e.g. fever,
157 acute allergic reaction, skin and mucosa abnormality, diarrhea, anorexia, vomiting,
158 nausea, muscle pain, headache, cough, fatigue) occurring on day 0~28. Data on
159 serious adverse events (SAEs) were collected throughout the trial. All AEs were
160 assessed for severity, and the relationship to vaccination was decided by investigators
161 before unblinding.

162 IMMUNOGENICITY

163 To assess immune response, blood samples were collected from each participant
164 different time points (0/28/42th day for Day 0,14 schedule, and 0/56th day for Day 0,28
165 schedule). The ability of the antibodies present in the blood sample to bind the
166 receptor binding domain (RBD) of SARS-CoV-2 was assessed by enzyme-linked
167 immunosorbent assay (ELISA). A dilution of 1:160 was considered as a positive
168 cutoff value. We also measured neutralizing antibody titer (Nab) using a modified
169 cytopathogenic effect assay. A titer of 1:8 or higher indicated seropositivity.
170 Seroconversion was defined as a change from seronegative (<1:8) to seropositive (\geq
171 1:8) or a 4-fold increase from baseline titers if seropositive.

172 The neutralizing antibody assay was performed by Chinese National Institutes for
173 Food and Drug Control, and the ELISA was performed by Sinovac Biotech.

174 NEGATIVE STAIN

175 Virus particles of vaccine used for phase 1 and 2 were diluted to a concentration of
176 0.04 mg/mL, deposited on a glow-discharged carbon-coated copper grid (Electron
177 Microscopy Sciences) and after 1 min, washed twice with buffer (20 mM Tris, 200
178 mM NaCl, pH 8.0), and stained with 1% phosphotungstic acid (pH 7.0) for 1 min.
179 Then the grid was imaged at room temperature using FEI Tecnai Spirit electron
180 microscope (Thermo Fisher Scientific) operated at an acceleration voltage of 120 kV.

181

182 STATISITICAL ANALYSIS

183 Safety evaluation was performed on participants who received at least 1 dose of the
184 vaccine or placebo by comparing the overall incidence rate of solicited and
185 unsolicited AEs among relevant groups. Immunogenicity assessment was performed
186 on the per-protocol set (PPS). The seroconversion rate was defined as a change from
187 seronegative to seropositive or a 4-fold increase from baseline titers if seropositive.
188 The titer distributions were described with reverse cumulative distribution curves and
189 were tested with the nonparametric Kruskal-Wallis test over the groups.

190 The Pearson Chi-square test or Fisher's exact test was adopted for the analysis of
191 binary outcomes. Clopper-Pearson method was used to compute the 95% confidence
192 intervals (CIs) of the binary outcome. ANOVA method was utilized to compare the
193 GMTs among groups. Hypothesis testing was two-sided with an alpha value of 0.05.
194 Analyses were conducted by SAS 9.4 (SAS Institute, Cary, NC, USA).

195 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	<i>P</i>
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/m ²)	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/m ²) §	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.

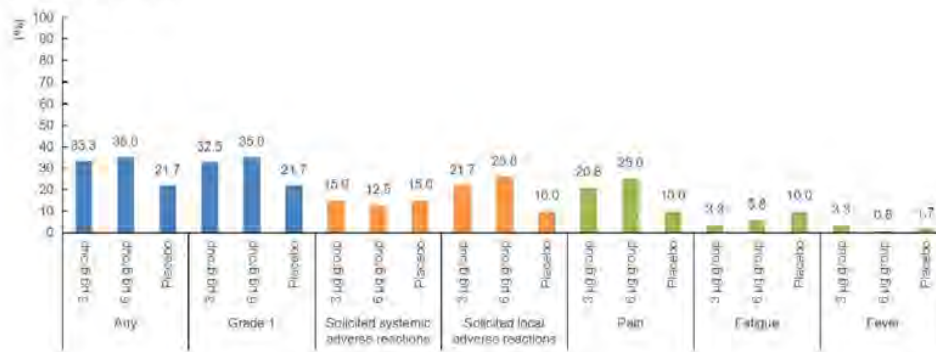
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212 ADVERSE REACTIONS

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).

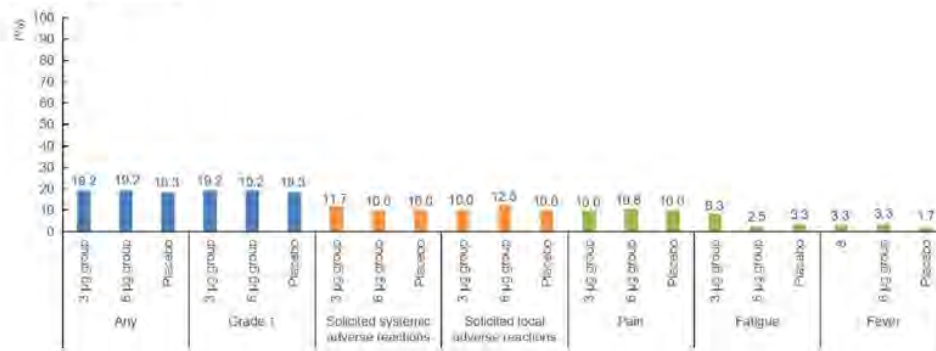
223 We did not observe any Grade 3 adverse reaction. Most reported adverse reactions
 224 resolved within 72 hours after vaccine administration. During the follow-up period, 3
 225 SAEs were reported from 3 subjects and neither was vaccine related.

A. Day 0,14 Schedule



226

B. Day 0,28 Schedule



227

228 **Figure legends**

229 **Figure 1. Incidence rates of adverse reactions among different groups in phase 2.**

230 (A) The incidence rates of adverse reactions among different groups with a Day 0,14 schedule. (B)

231 The incidence rates of adverse reactions among different groups with a Day 0,28 schedule.

232

233 **IMMUNOGENICITY**

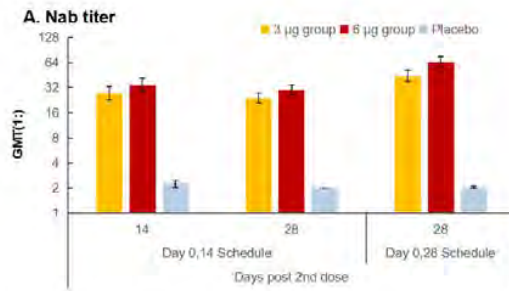
234 At baseline, all the 600 subjects were seronegative (with Nab titers of <1:8); but the

235 seroconversion rates increased over 90% during the later stages of the trial. Within

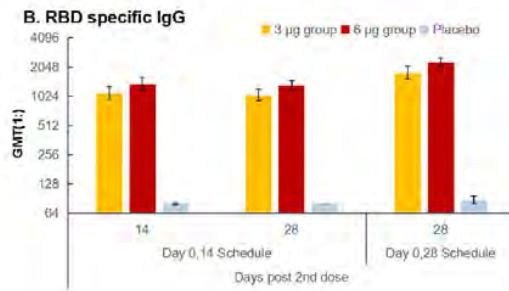
236 each dosage, there was no significant difference in the seroconversion rates between
237 Day 0,14 and Day 0,28 schedule. For the antibody response against the receptor
238 binding domain, similar results were observed (Table S2). No changes in
239 seropositivity frequencies and GMTs from baseline were found for the placebo group.

240 For subjects on Day 0,14 schedule, the GMT increased to 34.5 (95% CI, 28.5 to 41.8)
241 and 27.6 (95% CI, 22.7 to 33.5) in 6 µg and 3 µg group, respectively, and remained
242 stable after 28 days from the second injection (Fig. 2A). The neutralizing antibody
243 titers for subjects on Day 0, 28 schedule increased significantly 28 days after the
244 second injection, when compared to those of subjects on Day 0,14 schedule within
245 each dosage group. Almost similar trends like those observed for the neutralizing
246 antibody were observed during the evaluation of the IgG antibody level (Fig. 2B). In
247 addition, the neutralizing antibody titers significantly decreased with increasing age
248 (Fig. 2C and 2D); younger subjects tended to have a higher level of neutralizing
249 antibody titers .

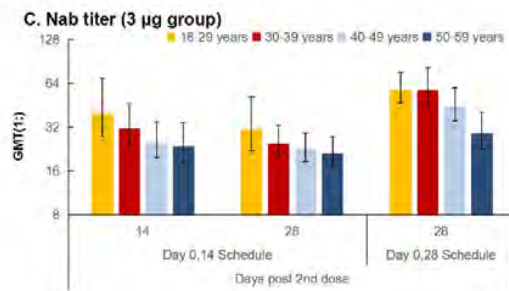
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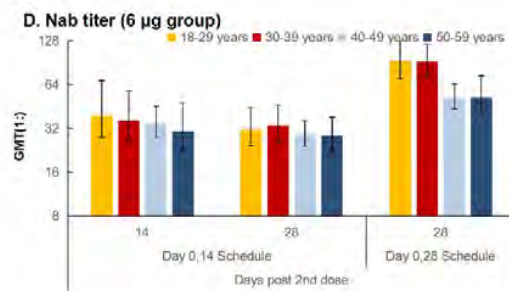
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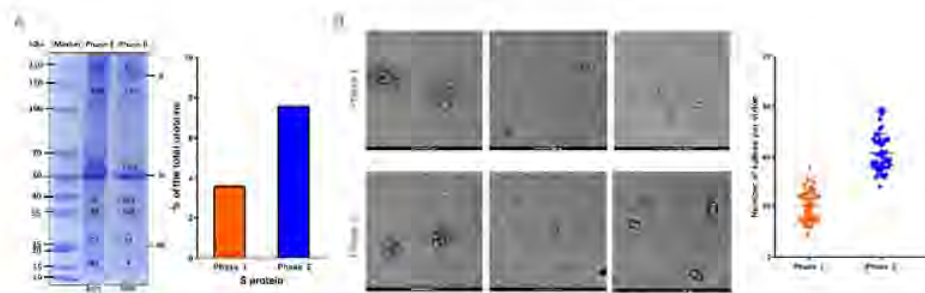


254 **Figure legends**

255 **Figure 2. Antibody Response in the Per-Protocol Cohort.**

256 (A) The neutralizing antibody titer in all participants 14 and 28 days after second dose in Day 0,14
 257 schedule and 28 days after second dose in Day 0,28 schedule. (B) The RBD specific IgG antibody
 258 titer in all participants 14 and 28 days after second dose in Day 0,14 schedule and 28 days after
 259 second dose in Day 0,28 schedule. (C) The neutralizing antibody titer among different age-groups
 260 at different time points from all participants that received 3 μg vaccine. (D) The neutralizing
 261 antibody titer among different age-group at different time points from all participants that received
 262 6 μg vaccine.

263



264

265 **Figure legends**

266 **Figure 3. The proportion of Spikes in CoronaVac used for phase 1 and 2 vaccine evaluation.**

267 (A) Protein composition analysis of CoronaVac samples from phase I and II by a NuPAGE 4-12%
 268 Bis-Tris gel, followed by whole-gel protein staining using Coomassie Blue gel staining reagent
 269 (45% methanol, 10% glacial acetic acid, 0.25% Coomassie Blue R-250). The viral protein bands
 270 of vaccine strain used for phase I and II were quantified by densitometry using ImageJ software
 271 with values depicted in the gel. The proportions of spikes to the total proteins in each gel lane in
 272 CoronaVac samples used for phase 1 and 2 were calculated separately. (B) Representative
 273 negative staining images of the CoronaVac samples used in phase 1 and 2 trials. Three images
 274 were randomly selected for each phase. Grouped scatter plot showing the numbers of Spikes on
 275 two-dimensional projections of randomly selected 50 virions of CoronaVac samples used for
 276 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 CO₂/O₂ 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

301 intact spike protein (~180 kDa) when compared to the Cell Factory-yielded
302 CoronaVac (Fig. 3A). Quantitative analysis showed that the intact spike protein
303 accounted for ~7% and ~ 3.7 of total protein mass used in phase 1 and 2 trials,
304 respectively. Electron microscopic examination of the samples further verified that the
305 average number of spikes per virion of the viral sample used in phase 2 trial was
306 almost double to those used in phase 1 trial (Fig. 3B). These observations indicated
307 that CoronaVac used in phase 2 trial contained more *bona fide* immunogens, which
308 explains its better protective immune responses, highlighting the importance of
309 developing an optimum manufacturing process and the integration of
310 multiple-disciplinary techniques, such as genomics and structural biology to support a
311 new era of precision vaccinology.

312 After two-dose vaccination, immune responses induced by Day 0,28 schedule was
313 above the value of Day 0,14 schedule regardless of the dosage of the vaccine, which
314 was consistent with our anticipation. By using Day 0,14 schedule, antibody response
315 could be induced within a relatively short time period, and this schedule could be
316 introduced to an emergency use and is of vital importance to handle COVID-19
317 pandemic situation. Regarding the Day 0,28 schedule, robust antibody response is
318 generated and longer persistence could be expected, which supports the need for a
319 routine use under the low incidence rate of COVID-19.

320 Nabs play an important role in virus clearance and have been considered as a key
321 immune correlate for protection or treatment against viral diseases. Twenty-eight days
322 after the two-dose vaccination, the Nab levels of individual schedules range from 23.8
323 to 65.4 in phase 2, which was lower than those of convalescent patients tested by the
324 same method in the same laboratory, of which the Nab average level was 163.7.¹¹ We

Article

325 assume the antibody level could provide satisfying protection against COVID-19
326 disease based on three reasons. Firstly, most of the surrogate endpoints based on
327 neutralizing antibodies ranges from 8-24, such as EV71 and Varicella vaccines.^{12,13}
328 Secondly, experience from our preclinical study indicated that the neutralizing
329 antibody titers of 1:24 elicited in macaques models conferred complete protection
330 against SARS-CoV-2. Thirdly, several studies revealed that antibody responses
331 generated from natural infection may decreased significantly, such as SARS-Cov-2,
332 SARS-CoV and MERS-CoV,¹⁴⁻¹⁶ however, recrudescence of these patients has been
333 rarely reported, which indicated that the immunological memory might play an
334 important role of prevention of re-infections.

335 Moreover, one prospective goal of our preclinical study and clinical trials was to
336 establish a vaccine-induced surrogate of protection. Compared with vaccine inducing
337 high level antibody, those inducing lower antibody level are more likely to produce
338 evidence on surrogate of protection. Under above assumptions, the dosage of 3 µg
339 with Day 0,14 or Day 0,28 schedule is adopted in our phase 3 trial.

340 When comparing antibody levels between age-groups, it should be noted that the
341 neutralizing antibody titers significantly decreased with increasing age. These results
342 are consistent with epidemiological trends observed in COVID-19 patients; those with
343 moderate or severe symptoms tend to be elderly.¹⁷ These results suggest that escalated
344 dosage or extra dose of CoronaVac might be needed in elderly.

345 Several limitations of this trial should be noted. Firstly, we only assessed the humoral
346 immunity in phase 2 trial, and more evaluation focus on response of Th1 and Th2 is
347 ongoing. Secondly, we only reported immune response data on healthy adults, and do

348 not include data on more susceptible populations, such as elderly or with comorbidity;
349 and also the immune persistence is not available yet, which need to be further studied.
350 Thirdly, we didn't compare the neutralizing antibody titers induced by CoronaVac and
351 convalescent COVID-19 patients in parallel, however, we conducted this detection of
352 convalescent serum specimens with same procedure performed in this phase 2 trial.
353 In conclusion, favorable safety and immunogenicity of CoronaVac was demonstrated
354 on both schedules and both dosages in this phase 2 clinical trial, which support the
355 conduction of phase 3 trial with optimum schedule/dosage per different scenarios.
356 Currently, our first priority is to evaluate the protective efficacy of the 3 µg dosage
357 under Day 0,14 schedule. Moreover, Day 0,28 schedule with 3 µg vaccine will also be
358 adopted in our future phase 3 clinical trials.

359

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2. It fights coronavirus variants

2.1. CoronaVac boosts immunity against variants of those who already had Covid-19, Chinese study shows

A paper published in the journal *Emerging Microbes and Infections* showed that, in unvaccinated individuals who had recovered from Covid-19 for 12 months, immunization with CoronaVac induced an increase in antibody levels and high neutralizing activity against the delta, alpha and beta variants of the SARS-CoV-2 virus. The study was published earlier this month and was conducted by scientists at the Clinical Research Center for Infectious Diseases, Shenzhen, China.

The study included 22 convalescent Covid-19 patients who received an inactivated virus vaccine one year after recovery, with 13 taking CoronaVac. They were followed during three phases: (a) one month after infection, (b) one year after recovery, before vaccination, and (c) two weeks to three months after immunization.

The researchers assessed the duration of immunity acquired by the infection and vaccine efficacy. After 12 months of recovery, the

volunteers' IgG antibody level had dropped by 23.7%, and the virus' neutralizing ability was also reduced, especially against variants.

With CoronaVac, there was a fourfold increase in IgG antibodies, reaching levels similar to those seen one month after infection. Moreover, neutralizing activity against the original strain and its variants increased from seven to 17-fold.

“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,” the authors stated.

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The SARS-CoV-2 inactivated vaccine enhances the broad neutralization against variants in individuals recovered from COVID-19 up to one year

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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.

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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

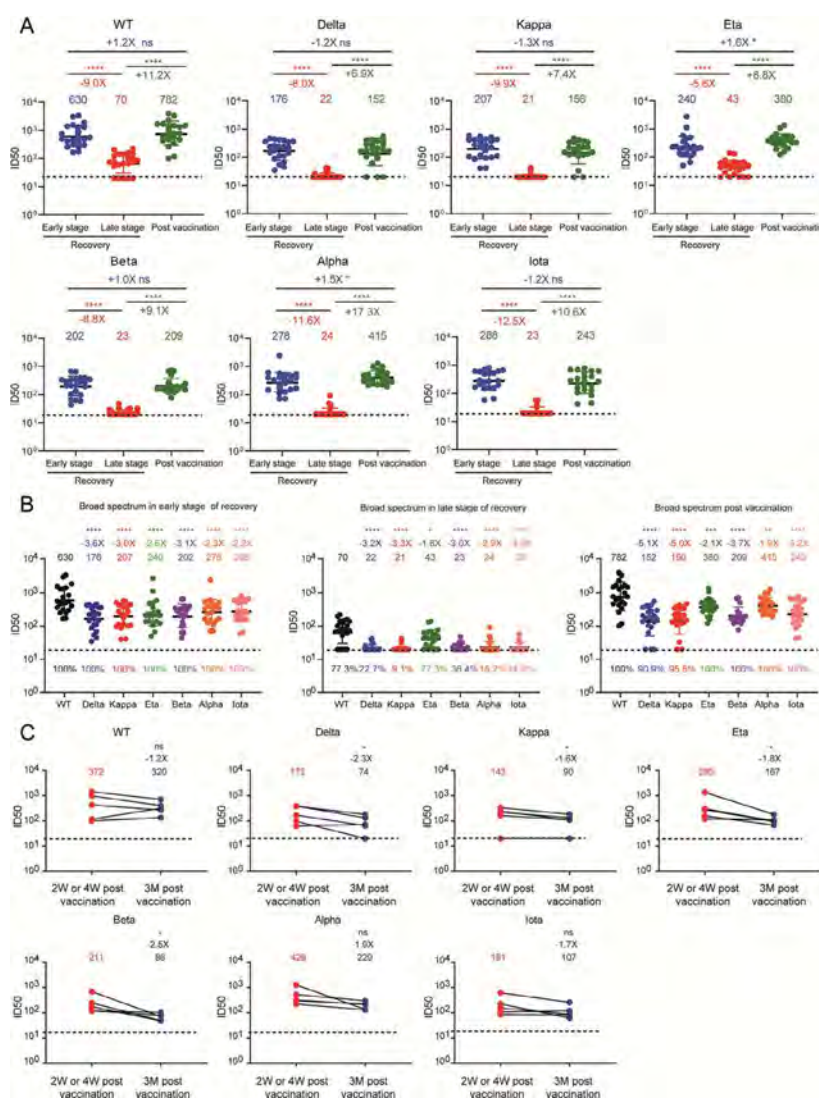


Figure 1. Neutralizing activities against WT SARS-CoV-2 and variants in convalescent individuals received the inactivated vaccine. (a) Plasma neutralizing activities against each SARS-CoV-2 strain in three follow-up time points were measured and shown in the values of 50% inhibition dilution (ID₅₀). (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 ID₅₀ 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.

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Disclosure statement

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

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Data availability statements

We are happy to share reagents and information in this study upon request.

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2.2. CoronaVac booster dose is effective against omicron, Chilean study finds

A phase 3 clinical study published on the MedRxiv platform showed that the third dose of CoronaVac, a vaccine from Butantan and Sinovac, significantly increases T cell response and the amount of neutralizing antibodies capable of recognizing the omicron and delta variants of SARS-CoV-2. The research was coordinated by the Pontifical Catholic University of Chile and the Millennium Institute on Immunology and Immunotherapy, which had already released preliminary results of the study in December 2021.

The researchers included 186 volunteers in the study who received a booster dose of the Butantan vaccine six to eight months after the second dose. During this period, the level of neutralizing antibodies, which was at 124.8 GMU (geometric mean units) one month after the second dose, was reduced to 39 GMU – a factor also observed in other vaccines, justifying the need for a booster.

With the third dose of CoronaVac, neutralizing antibodies jumped to 499 GMU, a 12-fold increase. By analyzing the neutralizing ability against omicron and delta in 30 of the volunteers, the scientists

identified neutralizing antibody mean titer of 50.7 against omicron and 159.2 against delta, and the seropositivity rate (antibody production) was 76.7% and 93%, respectively.

Cellular immune response, more specifically CD4+ T cell activity, was assessed in 40 of the participants. “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,” the study authors state. T cells also showed a good response to omicron and delta, similar to the response against the original strain of SARS-CoV-2.

In an analysis released in October 2021 by the Chilean Ministry of Health, the vaccine’s protection against Covid-19 hospitalizations in the country increased from 84% to 88% after the booster dose. Other studies have shown that the CoronaVac booster dose boosts immune response, including against coronavirus variants.

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1 **A booster dose of an inactivated SARS-CoV-2 vaccine increases**
2 **neutralizing antibodies and T cells that recognize Delta and**
3 **Omicron variants of concern.**

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39 CoronaVac; Phase 3 clinical trial; SARS-CoV-2; COVID-19; inactivated vaccine,
40 Booster dose.

41

42 **Abstract**

43 **Background:** CoronaVac® is an inactivated SARS-CoV-2 vaccine approved by the
44 World Health Organization. Previous studies reported increased levels of
45 neutralizing antibodies and specific T cells two- and four-weeks after two doses of
46 CoronaVac®, but the levels of neutralizing antibodies are reduced at six to eight
47 months after two doses. Here we report the effect of a booster dose of CoronaVac®
48 on the anti-SARS-CoV-2 immune response generated against variants of concern
49 (VOC) Delta and Omicron in adults participating in a phase 3 clinical trial in Chile.

50 **Methods:** Volunteers immunized with two doses of CoronaVac® in a four-week
51 interval received a booster dose of the same vaccine between twenty-four and
52 thirty weeks after the 2nd dose. Four weeks after the booster dose, neutralizing
53 antibodies and T cell responses were measured. Neutralization capacities and T
54 cell activation against VOC Delta and Omicron were detected at four weeks after
55 the booster dose. **Findings:** We observed a significant increase in neutralizing
56 antibodies at four weeks after the booster dose. We also observed an increase in
57 CD4⁺ T cells numbers over time, reaching a peak at four weeks after the booster
58 dose. Furthermore, neutralizing antibodies and SARS-CoV-2 specific T cells
59 induced by the booster showed activity against VOC Delta and Omicron.

60 **Interpretation:** Our results show that a booster dose of CoronaVac® increases the
61 anti-SARS-CoV-2 humoral and cellular immune responses in adults. Immunity
62 induced by a booster dose of CoronaVac® is active against VOC, suggesting an
63 effective protection.

64

65 **Background**

66 The ongoing pandemic caused by severe acute respiratory syndrome
67 coronavirus-2 (SARS-CoV-2) has promoted the rapid development of safe,
68 immunogenic, and effective vaccines against SARS-CoV-2 to be used by the
69 general population, which have successfully reduced the transmission of the
70 disease burden. CoronaVac® is an inactivated SARS-CoV-2 vaccine developed by
71 Sinovac Life Sciences Co., Ltd. (Beijing, China) and is among the current vaccines
72 approved by the World Health Organization (WHO) to combat coronavirus disease
73 2019 (COVID-19) and one of the most used vaccines worldwide^{1,2}. Phase I/II
74 clinical trials in China demonstrated that this vaccine induces cellular and humoral
75 response upon immunization³⁻⁵. Furthermore, the ongoing phase 3 clinical trial in
76 Chile has described an increase in the levels of IgG and neutralizing antibodies in
77 adults aged 18-59 years and ≥ 60 years two- and four-weeks after the second dose
78 of CoronaVac®⁵⁶. In addition, this vaccination promotes the activation of a T cell
79 immune response against SARS-CoV-2 antigens in a 0-14 immunization schedule
80⁵ (two-weeks interval), being an effective vaccine to prevent COVID-19^{7,8}. In Chile,
81 93.7% of the target population has received a first vaccine dose, and 91.4% were
82 fully vaccinated with CoronaVac® on December 10th of 2021 in a 0-28 days
83 vaccination schedule⁹. Although this primary immunization schedule induces
84 neutralizing antibody present in the serum of vaccinated people¹⁰, these titers
85 decrease in time^{6,11,12} and have lower levels of neutralization against highly
86 transmissible variants of concern (VOC) as compared to the original vaccine strain,
87 potentially decreasing the effectiveness of these vaccines as new variants emerge

88 ^{13–17}. For these reasons, the use of booster doses was approved in Chile in August
89 2021 in Chile for high-risk populations and adults at five months after
90 administration of the second dose ¹⁸. In this sense, a report published in October
91 2021 in Chile, showed that the effectiveness of CoronaVac[®] against COVID-19
92 increase from 56% to 80% fourteen days after the application of the booster dose
93 ¹⁹. Notably, a previous study performed in adults aged 18-59 years old
94 demonstrated that a booster dose of CoronaVac[®], applied six months after the first
95 dose to individuals that previously received two doses of this vaccine, increased
96 the levels of antibodies 3-5-fold as compared to the levels observed four weeks
97 after the second dose ¹². Here, we further extend these findings by reporting the
98 levels of neutralizing antibodies and specific T cells against SARS-CoV-2 and its
99 activity against VOC Delta and Omicron in adults ≥ 18 years old that participated in
100 the phase 3 clinical trial carried out in Chile, who were vaccinated in a 0-28-days
101 vaccination schedule and received a booster dose five months after the second
102 dose.

103

104 **Materials and methods**

105 **Volunteers and sample collection**

106 Blood samples were obtained from volunteers recruited in the clinical trial
107 CoronaVac03CL (clinicaltrials.gov #NCT04651790) carried out in Chile starting in
108 November 2020. The Institutional Scientific Ethical Committee of Health Sciences
109 reviewed and approved the study protocol at the Pontificia Universidad Católica de
110 Chile (#200708006). Trial execution was approved by the Chilean Public Health
111 Institute (#24204/20) and was conducted according to the current Tripartite

112 Guidelines for Good Clinical Practices, the Declaration of Helsinki ²⁰, and local
113 regulations. Informed consent was obtained from all volunteers upon enrollment.
114 Volunteers receive two doses of CoronaVac[®] (3 µg or 600SU of inactivated SARS-
115 CoV-2 inactivated along with alum adjuvant) in a four-week interval (0-28-day
116 immunization schedule) and then a booster dose five months after the second
117 dose. A complete inclusion and exclusion criteria list have been reported ⁵. On
118 November 11th, 2021, one hundred and eighty-six volunteers in the immunogenicity
119 branch received the booster dose. The antibody and cell mediated immune
120 responses were evaluated volunteers that had completed all their previous visits in
121 one of the centers of the study (**Figure 1A**). Blood samples were obtained from all
122 the volunteers before administration of the first dose (pre-immune), two, four, and
123 twenty weeks (or five months) after the second dose, and four weeks after the
124 booster dose (**Figure 1B**).

125 **Procedures**

126 The presence of antibodies against RBD with neutralizing capacities were
127 measured in sera from seventy-seven volunteers that had completed all their study
128 visits, including one month after the booster dose of CoronaVac[®]. The neutralizing
129 capacities of circulating antibodies were evaluated by a surrogate virus
130 neutralization test (sVNT) (Genscript Cat#L00847-A). Samples were two-fold
131 serially diluted starting at a 4-fold dilution until reaching a 512-fold dilution. Assays
132 were performed according to the instructions of the manufacturer and as reported
133 previously ⁵. Neutralizing antibody titers were determined as the last fold dilution in
134 which the interaction between hACE2 and RBD was inhibited by 30% or more.
135 Samples with a percentage of inhibition $\leq 30\%$ at the lowest dilution (1:4) were

136 assigned as seronegative with a titer of 2. A sample was considered seropositive
137 when its titer was higher than the pre-immune titer. The percentage of inhibition
138 was determined as: $100 * [\text{OD}_{450\text{nm}} \text{ value of negative control} - \text{OD}_{450\text{nm}} \text{ value of}$
139 $\text{sample}] / [\text{OD}_{450\text{nm}} \text{ of negative control}]$. A standard curve was used to plot the
140 neutralization response in the samples as international units (IU) using the WHO
141 International Standard for SARS-CoV-2 antibody (NIBSC code 20/136), which was
142 prepared according to the instructions of the manufacturer ²¹. Data were analyzed
143 using a sigmoidal curve model with a logarithm transformation of the concentration,
144 and the final concentration for each sample was the average of the product of the
145 interpolated IU from the standard curve and the sample dilution factor required to
146 reach the OD450 value that falls within the linear range determined for each
147 sample. Samples with undetermined concentration at the lowest dilution tested
148 (1:4) were assigned to the lower limit of quantification (16.4 IU). The Geometric
149 Mean Units (GMU) and titers (GMT) were represented in **Figure 2** and
150 **Supplementary Figure 1**, respectively. **Table 2** shows comparisons among the
151 visits.

152 Conventional virus neutralization tests (cVNT) were performed in sixty-two
153 of the previous seventy-seven volunteers and evaluated as previously reported ⁵.
154 Briefly, Vero E6 cells were infected with a SARS-CoV-2 strain obtained by viral
155 isolation in tissue cultures (33782CL-SARS-CoV-2 strain, D614G variant).
156 Neutralization assays were carried out by the reduction of cytopathic effect (CPE)
157 in Vero E6 cells (ATCC CRL-1586). The titer of neutralizing antibodies was defined
158 as the highest serum dilution that neutralized virus infection, at which the CPE was
159 absent as compared with the virus control wells (cells with CPE). Vero E6 cells

160 were seeded in 96-well plates (4×10^4 cells/well). For neutralization assays, 100 μ L
161 of 33782CL-SARS-CoV-2 (at a dose of 100 TCID₅₀) were incubated with serial
162 dilutions of heat-inactivated sera samples (dilutions of 1:4, 1:8, 1:16, 1:32, 1:64,
163 1:128, 1:256, and 1:512) from participants for 1h at 37 °C. Cytopathic effect on
164 Vero E6 cells was analyzed 7 days after infection.

165 A pseudotyped virus neutralization test (pVNT) assay was performed to
166 assess the capacity of the antibodies against SARS-CoV-2 VOC in samples from
167 thirty volunteers of the seventy-seven previously analyzed by sVNT. As previously
168 reported ¹⁴, a HIV-1 backbone expressing firefly luciferase as a reporter gene and
169 pseudotyped with the SARS-CoV-2 spike glycoproteins (HIV-1-S Δ 19) from from
170 lineage B.1 (D614G) or variants Delta (T19R, del157/158, L452R, T478K, D614G,
171 P681R, D950N) and Omicron (A67V, Δ H69-V70, T95I, Y145D, Δ G142 -V143-
172 Y144, Δ N211, EPE 213-214, G339D, S371L, S373P, S375F, K417N, N440K,
173 G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, T547K, D614G,
174 H655Y, N679K, P681H, N764K, N865K, Q954H, N969K, L981F) was prepared as
175 previously described ²². Serum samples were two-fold diluted starting at 1:10 or 1:4
176 and the estimation of the ID80 was obtained using a 4-parameter nonlinear
177 regression curve fit measured as the percent of neutralization determined by the
178 difference in average relative light units (RLU) between test samples and
179 pseudotyped virus controls. Also, cVNT assays were performed to assess the
180 capacity of the antibodies against SARS-CoV-2 Delta variant in samples from
181 nineteen volunteers of the seventy-seven previously analyzed by sVNT.

182 ELISPOT and flow cytometry assays were performed to evaluate the cellular
183 immune response in forty volunteers of the seventy-seven previously analyzed

184 **(Figure 1A)**, stimulating PBMCs with four Mega Pools (MPs) of peptides derived
185 from the proteome of SARS-CoV-2 ²³: peptides from the S protein of SARS-CoV-2
186 (MP-S), the remaining proteins of the viral particle (excluding S protein peptides)
187 (MP-R), and shorter peptides from the whole proteome of SARS-CoV-2 (MP-CD8-
188 A and MP-CD8-B) ²³. Thirty of the previously analyzed volunteers were also
189 stimulated with three Mega Pools (MP) of VOC, provided by La Jolla Institute for
190 Immunology. MP derived from the S protein of SARS-CoV-2 WT, SARS-CoV-2
191 B1.617.2 MP 4326 (Delta variant), and SARS-CoV-2 B1.1.529 MP 4359 (Omicron
192 variant) ¹⁴ were used to evaluate T cell activation at four weeks after the booster
193 dose. Positive and negative controls were held for each assay. The number of Spot
194 Forming Cells (SFC) for interferon gamma (IFN- γ) were determined by ELISPOT
195 and the expression of Activation-Induced Markers (AIM) by T cells was evaluated
196 by flow cytometry. Assays were performed according to the instructions of the
197 manufacturer and as reported previously ⁵. Further details on the ELISPOT assay,
198 antibodies used for flow cytometry, and the respective protocols can be found in
199 the Supplementary information and **Supplementary Table 1**.

200 Interleukin 2 (IL-2) and IFN- γ secretion were evaluated in the supernatants
201 of twenty-two volunteers previously stimulated for 20h with SARS-CoV-2 MP of
202 peptides derived from the Spike protein of VOC, using a Luminex 200 xMap
203 multiplex system (Luminex Corporation, Austin, TX). The limit of detection for the
204 cytokine measured ranged from 4.2 to 13,390 pg/mL, according to manufacturer's
205 instructions. Further experimental details can be found in **Supplementary**
206 **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

254 was observed, as seen in **Figure 2C and F**, observing an increase in the level of
255 neutralizing antibodies evaluated by both techniques of more than 9-fold at four
256 weeks after the booster dose (GMU 300.5, 95% CI=203.5-443.6 and GMT 47.3,
257 95% CI=32.1-69.5) as compared to the response observed twenty weeks after the
258 second dose (GMU 32.4, 95% CI=25.1-41.8 and GMT 5.0, 95% CI=3.5-7.0).
259 Equivalent to the 18-59 years old group, the seropositivity rate in this age group
260 reached 100% four weeks after the booster dose (**Table 2**). The seropositivity rate
261 achieved at four weeks after the booster dose was the highest when compared
262 with the other visits in this study in the total vaccinated group and in both groups
263 analyzed.

264

265 **A booster dose of CoronaVac® induces a robust cellular immune**
266 **response in adults.**

267 The cellular responses following a booster dose of CoronaVac® were
268 evaluated in 40 volunteers. We observed that CD4⁺ T cell activation was increased
269 twenty weeks after the second dose as compared to the other time points in both
270 age groups, suggesting that CoronaVac® can stimulate CD4⁺ T cell responses that
271 are sustained over time (**Figure 3A-C**). Importantly, we observed a significantly
272 further increase in CD4⁺ T cell activation in both age groups following the booster
273 dose, as compared to the pre-immune sample and samples obtained at two and
274 four weeks after the second dose (**Figure 3A-C**). However, this difference was not
275 significant as compared to the sample obtained twenty weeks after the second
276 dose (**Figure 3A-C**). Moreover, we did not observe a significant increase in the
277 expression of AIM by CD8⁺ T cells following the booster dose as compared to any

278 other time point, suggesting that specific CD8⁺ T cell responses induced by
279 CoronaVac[®] are not detected with the current methodologies, even after a third
280 dose (**Supp. Figure 2A and C**). Accordingly, we observed an increase in IFN- γ
281 production upon stimulation with mega-pools of peptides (MPs) S and R by
282 ELISPOT at four weeks after the booster dose for both groups, as compared to the
283 pre-immune sample (**Figure 3D-F**). As in the case of flow cytometry, we did not
284 observe significant increase of IFN- γ ⁺ SFCs upon stimulation with CD8 MPs at any
285 time point (**Supp. Figure 2**). These results suggest that although humoral
286 responses decrease over time following vaccination with CoronaVac[®], CD4⁺ T cell
287 responses remain significantly increased as compared to pre-immune samples and
288 the booster dose promotes a small increase both IFN- γ production and CD4⁺ T cell
289 activation that is not significantly different as compared to the levels observed 20
290 weeks after the second dose.

291

292 **Neutralizing antibodies and specific T cells induced by a booster dose** 293 **of CoronaVac[®] recognize Delta and Omicron variants of SARS-CoV-2.**

294 As we observed that the neutralization capacity and the T cell responses
295 increased significantly with the booster dose and knowing that vaccinated
296 volunteers exhibit decreased neutralization against VOC¹⁴, we proceeded to
297 evaluate the neutralizing capacities of antibodies in the serum from thirty booster-
298 vaccinated individuals in pseudotyped virus neutralization test (pVNT) assay
299 against two variants of concern of SARS-CoV-2, comparing with the level obtained
300 for the D614G SARS-CoV-2 variant (B.1 lineage, **Figure 4A-B**). We observed that

301 the titers of antibodies with neutralizing capacities against Delta and Omicron
302 variant show a significant reduction as compared to the levels achieved for the
303 D61G variant (D614G: GMT 241.8, CI=155.7-375.6, Delta: 159.2, CI=99.1-256.0
304 and Omicron: GMT 50.7, CI=30.4-84.8), with a reduction of 1.5 for Delta and 4.8
305 for Omicron (**Figure 4A**). However, when we compared the changes in
306 seropositivity for Delta and Omicron (**Figure 4B**), we observed a 93% and 76.7%,
307 respectively, following the booster dose (**Table 3**). Neutralization assays against
308 Delta variant with a cVNT in a different group of nineteen volunteers also show that
309 antibodies induced four weeks after the booster dose have reduced capacity to
310 neutralize this VOC (**Supp Figure 4A**), although the seropositivity rate observed is
311 84% (**Supp Figure 4B**).

312 The cellular responses for VOC following a booster dose of CoronaVac®
313 were also evaluated in thirty volunteers using MPs of peptides derived from the
314 Spike protein of Delta and Omicron variants. We observed equivalent numbers of
315 AIM by CD4⁺ T cells after four weeks of the booster dose upon stimulation with MP-
316 S of SARS-CoV-2 WT, Delta, or Omicron variant (**Figure 5A**), with no significant
317 differences between the response against the MP-S of the variants as compared to
318 the MP-S of the WT strain. IFN- γ secreting T cells were also analyzed in these
319 samples and no differences were observed (**Figure 5B**). We also quantified the
320 production of different cytokines in the supernatant of PBMCs stimulated with the
321 MP-s of WT, Delta, and Omicron variants, observing that at four weeks after the
322 booster dose the stimulated cells secrete equivalent levels of IL-2 and IFN- γ
323 (**Figure Supp 3**). These results suggest that although the humoral response

324 measured as neutralization capacities and seroconversion against these VOC is
325 lower as compared to the humoral response against the D614G strain, the cellular
326 responses against SARS-CoV-2 VOC is equivalent to the responses elicited by the
327 wild type strain in volunteers vaccinated with booster dose.

328

329 **Discussion**

330 In this report we evaluated the humoral and cellular immune response
331 generated four weeks after the application of a booster dose of inactivated
332 CoronaVac® vaccine in a cohort of volunteers enrolled in the phase 3 clinical trial
333 held in Chile. The data reported here showed that although there was an adequate
334 humoral response after two doses of CoronaVac®, with a 65.9% of effectiveness in
335 preventing COVID-19⁸, both the sVNT and cVNT assays showed a decrease in
336 the GMT of neutralizing capacities of circulating antibodies against SARS-CoV2
337 twenty weeks after the second dose (**Figure 2**). Due to this decrease in
338 neutralizing capacities, a booster dose of CoronaVac® was evaluated in a clinical
339 study in China, showing promising results in enhanced humoral immune
340 responses^{12,26}. The evaluation of the immune response reported here shows that
341 after the booster dose, the neutralizing titers and seroconversion rates increase in
342 the whole group, to a higher extent than two weeks after the second dose, where
343 the peak in neutralization was previously observed, which is in line with the
344 observed by *Clemens et al.*,¹⁵. Also, we observed a steady activation of the CD4⁺
345 T cells and secretion of IFN- γ during the time-points evaluated (**Figure 3**).

346 Since the neutralizing antibody titers correlated with protection against
347 SARS-CoV-2 infection ¹⁰, these results likely imply a better outcome and protection
348 against COVID-19, as reported in previous studies performed in Israel that showed
349 a decrease in the transmission and the disease severity disease by this virus
350 twelve or more days after booster inoculation. In Chile, the effectiveness and
351 prevention in hospitalization increased when assessed fourteen days after the
352 booster dose of CoronaVac[®] ^{19,27}. Another study, performed with a booster dose of
353 CoronaVac[®], showed that an additional dose result in good neutralization capacity
354 against parental SARS-CoV-2 and against Delta variant four weeks after the
355 booster dose, generating a long-lasting humoral response, which was due to an
356 enhancement of the memory immune response generated by B cells ²⁶.

357 Adults ≥ 60 years old produced lower levels of antibodies with neutralizing
358 capacities than the whole group during this study, which was also described
359 previously (**Figure 2C and F**) ⁵. This result is in line with previous data reported for
360 a population vaccinated in Chile ⁶, among hospital workers who received two
361 doses of CoronaVac[®] ²⁸, and in a study with the mRNA-1273 vaccine ²⁹. In this
362 sense, our results are equivalent to those described in a phase I/II of the clinical
363 trial performed with CoronaVac[®] in China, showing that the neutralizing antibody
364 titers in this group decreased at five months after the second dose and that a
365 booster dose is required 6-8 months after the first vaccination to rapidly increase
366 and maintain the neutralizing antibody titers ³⁰.

367 In the case of the T cell response (**Figure 3**), other studies have shown that
368 Pfizer BNT162b2 and mRNA-1273 induce durable CD4⁺ T cell activation and
369 cytokine production up to six months following vaccination, but it remains to be

370 elucidated whether expression of AIM by CD4⁺ T cells and cytokine production
371 further increase following a booster dose with these vaccines ^{31,32}. Here, we
372 observed that the activation of CD4⁺ T cells and IFN- γ production stays increased
373 up to twenty weeks after the second dose, and after the booster dose both
374 parameters increased in the 18-59 years old group and was maintained at the
375 levels observed twenty weeks after the second dose in adults ≥ 60 years old. In
376 contrast to BNT162b2 and mRNA-1273 vaccines, CoronaVac[®] delivers not only
377 the Spike protein upon immunization but also other viral antigens, which may
378 explain why vaccinated individuals still display AIM⁺ CD4⁺ T cells five months after
379 the second dose, regardless of a third dose.

380 When the neutralization capacity analyzed by pVNT of the VOC Delta and
381 Omicron was evaluated four weeks after the booster dose, we observed
382 differences in the neutralization capacity as compared to the D614G variant
383 (lineage B.1), which does not exhibit mutations in the RBD of the S1 protein
384 (**Figure 4 and table 3**). We previously reported that CoronaVac[®] is able to induce
385 neutralization against the Delta variant at 4 weeks after the second dose, although
386 to a lesser extent compared to the WT strain ¹⁴. Another study recently reported a
387 significant increase in the neutralizing capacity after a booster dose with
388 CoronaVac[®] for the Delta variant, as compared to the levels observed in volunteers
389 vaccinated with two doses ^{26,33}. Although we did not observe similar levels of
390 enhanced neutralization against the variants Delta after the booster dose using
391 pVNT (**Figure 4A**), the seropositivity against Delta variant is almost 100% (**Figure**
392 **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 Omicron¹⁶. 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 Omicron¹⁵. 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}

417 and mRNA vaccines, which has been explained by the high conservation of T cell
418 epitopes. In this sense, the booster dose of CoronaVac[®] induces the expression of
419 CD4⁺ T cell activation markers and secretion of IFN- γ and IL-2 against the VOC
420 Delta and Omicron, which is comparable to the response generated against the
421 WT strain (**Figure 5**). In line with this, a recent study has shown that T cell
422 responses against the ancestral strain are cross-reactive against the Omicron
423 variant in convalescent individuals and volunteers vaccinated with Pfizer
424 BNT162b2 ³⁸, supporting the idea that the induction of T cell responses against the
425 ancestral strain may be protective against the Omicron variant.

426 Our report shows that the booster dose of CoronaVac[®] in a 0-28 days
427 schedule induces antibodies with neutralizing capacities, which are higher than the
428 levels observed at 2- and 4-weeks after the second dose, generating an increased
429 humoral response even in adults ≥ 60 years old. Besides this, our results suggest
430 that a third dose of CoronaVac[®] supports CD4⁺ T cell activation, which may confer
431 either protection or enhanced immune responses against the virus and prevent
432 severe disease following exposure to SARS-CoV-2 exposure. Importantly, the
433 humoral and cellular immune response promoted by a booster dose of CoronaVac
434 shows activity against Delta and Omicron variants and probably results in better
435 effectiveness of this vaccine during predominance of these VOC.

436

437 **Limitations**

438 This study presents several limitations, such as the reduced sample size for
439 the assays and the absence of data for neutralization against Omicron variant with

440 a conventional viral neutralization test. The assessment of total antibody response
441 against Spike proteins and other SARS-CoV-2 proteins would also add additional
442 information about the humoral immune response against SARS-CoV-2 after the
443 booster dose. Due to the limit of quantification of the technique, samples with
444 undetermined concentration at the lowest dilution tested (1:4) were assigned the
445 lower limit of quantification (16.4 IU) and other neutralization assays.

446

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459 **Competing interests**

460 ZG and MW are SINOVAC Biotech employees and contributed to the
461 conceptualization of the study (clinical protocol and eCRF design) and did not
462 participate in the analysis or interpretation of the data presented in the manuscript.

463 A.S. is a consultant for Gritstone, Flow Pharma, Arcturus, Immunoscope,
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465 has filed for patent protection for various aspects of T cell epitope and vaccine
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489 Note: Members of the CoronaVac03CL Study Group are listed in the

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491

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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

707 percentage of activated AIM⁺ CD4⁺ T cells (OX40⁺, CD137⁺) were determined upon
708 stimulation for 24h with MP-S+R in samples obtained at pre-immune, two weeks
709 after the second dose, four weeks after the second dose, twenty weeks the second
710 dose, and four weeks after the booster dose. Data from flow cytometry was
711 normalized against DMSO and analyzed separately by a Friedman test against the
712 booster dose. Results were obtained from a total of forty volunteers (**A**), twenty-
713 one were of them were adults between 18-59 years old (**B**), and nineteen of them
714 were ≥ 60 years old (**C**). Changes in the secretion of IFN- γ were quantified as the
715 number of Spot Forming Cells (SFCs) in peripheral blood mononuclear cells of
716 volunteers that received a booster dose of CoronaVac® 20 weeks after the second
717 dose. **D-F**. Data was obtained upon stimulation with MP-S+R for 48h in samples
718 obtained at pre-immune, two weeks after the second dose, four weeks after the
719 second dose, twenty weeks the second dose, and four weeks after the booster
720 dose. Results were obtained from a total of 40 volunteers (**D**), 21 were of them
721 were adults between 18-59 years old (**E**), and 19 of them were ≥ 60 years old (**F**).
722 Data from ELISPOT were analyzed separately by Friedman test against the
723 booster dose * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$; **** $p < 0.0001$.

724

725 **Figure 4: Quantification of circulating neutralizing antibodies against SARS-**
726 **CoV-2 variants in volunteers that received the booster dose of CoronaVac®.**

727 **A.** Neutralizing antibodies were detected in the serum of thirty volunteers, four
728 weeks after the booster dose of CoronaVac®, using a pseudotyped virus
729 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



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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

760 **Table**

761 **Table 1: Demographic and clinical data of seventy-seven volunteers**
 762 **analyzed.**

763
 764

	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)

765 *Arterial hypertension: AHT; Chronic obstructive pulmonary disease: COPD; Mellitus diabetes: MD;*
 766 *Hypothyroidism: HT; Allergic rhinitis: AR*
 767

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	2 nd dose + 2 weeks	2 nd dose + 4 weeks	2 nd dose + 20 weeks	3 rd 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

771 *svNT: Surrogate Virus Neutralization; cVNT: Conventional Virus Neutralization; GMT: Geometric*
 772 *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

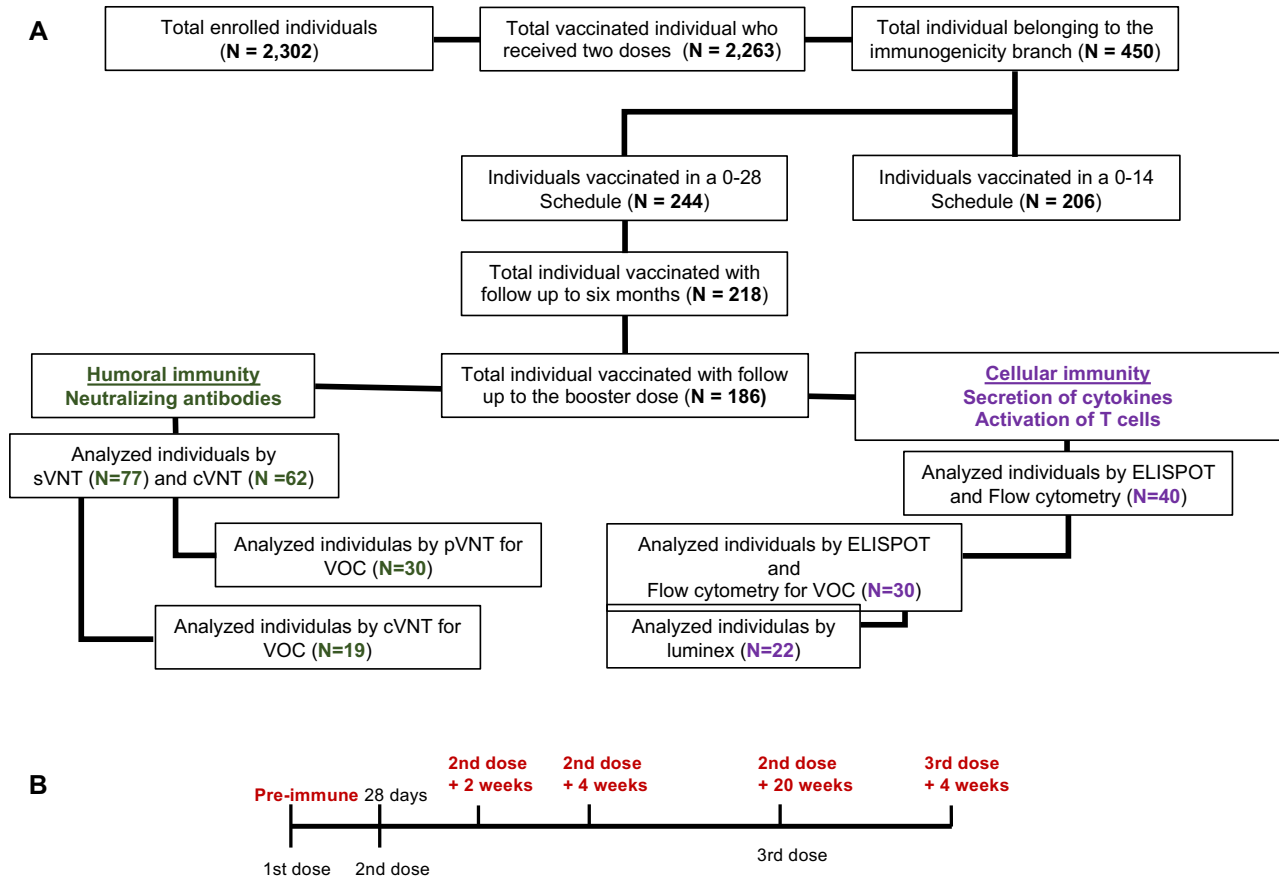


Figure 2

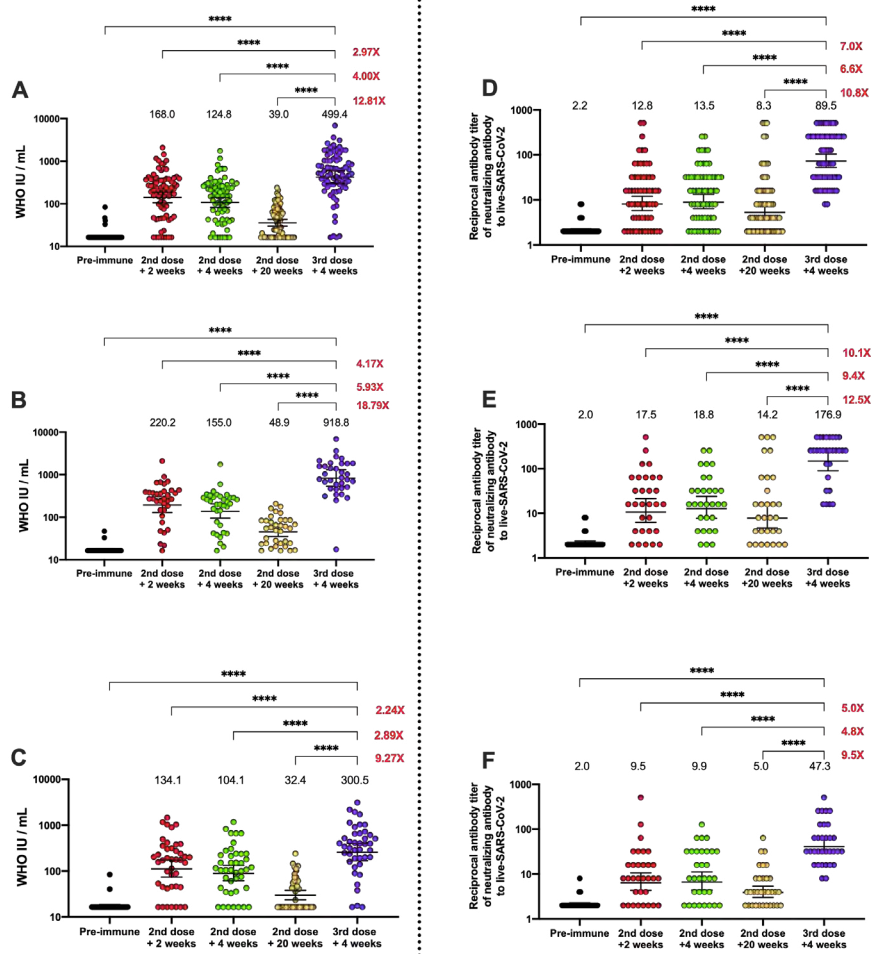


Figure 3

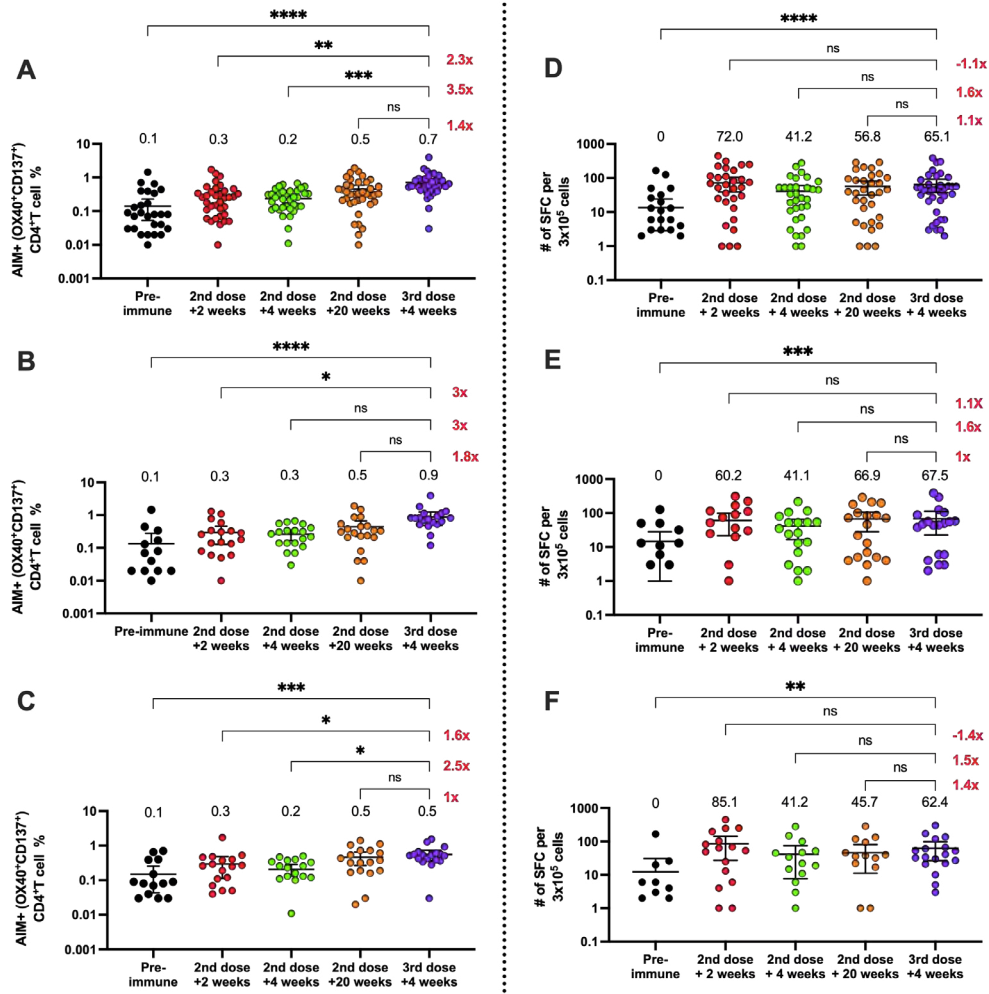


Figure 4

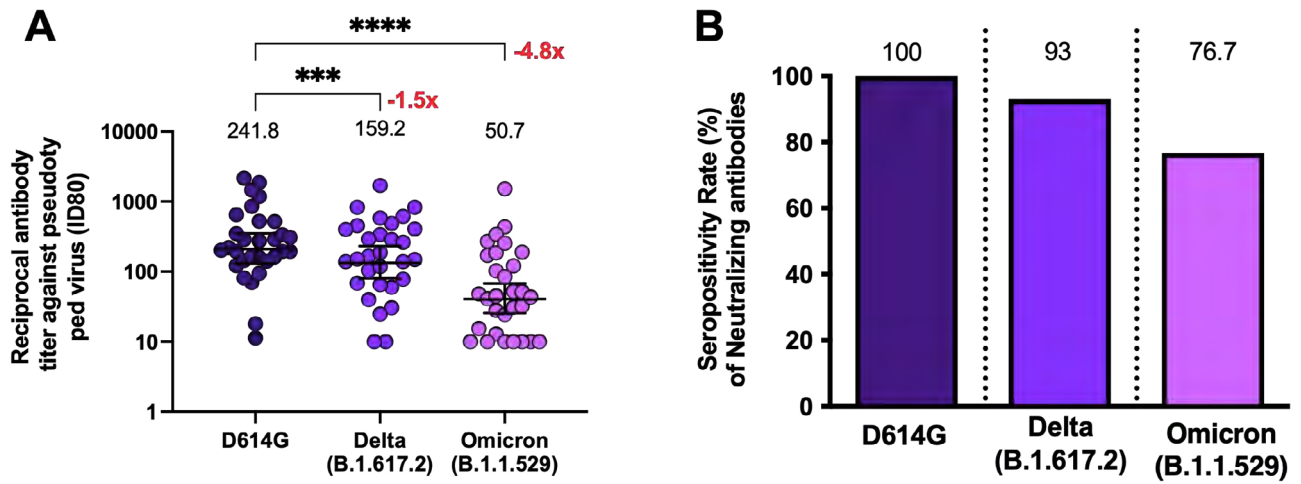
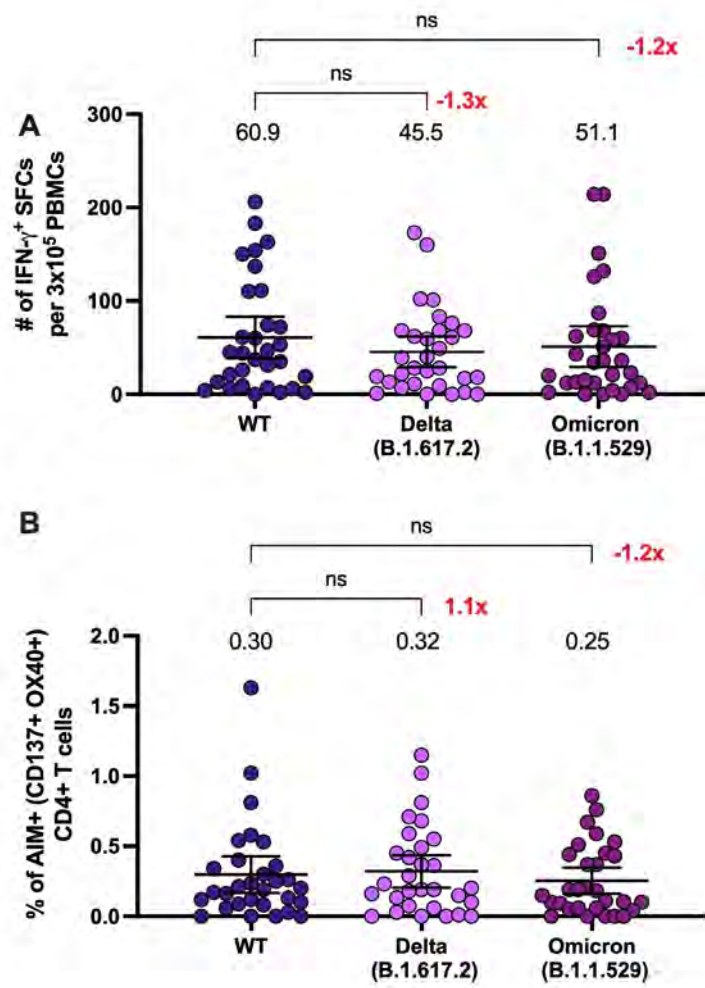


Figure 5



2.3. Chinese study proves effectiveness of CoronaVac against severe cases of the delta variant

A research published in the Annals of Internal Medicine journal showed that the vaccines of inactivated virus such as CoronaVac presents a high effectiveness against the delta variant of SARS-CoV-2, protecting against severe cases during the circulation of delta between May and June of 2021 in Guangdong, in China.

The scientists assessed 10.805 adult patients that were diagnosed with Covid-19, divided into three groups: non vaccinated, vaccinated with one dose and fully immunized (two doses) with the vaccines of inactivated virus most used in China - CoronaVac (applied on about 60% of the participants) and HB02/ Sinopharm (applied on about 40%). Afterwards, they estimated the effectiveness of the vaccines against the infection, against symptomatic cases, against pneumonia and against the severe disease.

On individuals with a complete vaccinal scheme, the effectiveness was 52% against infections, 60% against symptomatic cases, 78% against pneumonia and 100%

against severe cases of Covid-19. And, among the partially immunized, the vaccines provided a protection of 10,7% against infections, 6,8% against symptomatic cases and 11,6% against pneumonia.

The results highlight the efficacy in the real world of inactivated virus vaccines, confirming the findings of other studies of effectiveness already published, such as Project S from Butantan and a Chilean research with ten million people, that evaluated CoronaVac. “Besides, the research reinforces the importance of both doses, showing that the partial vaccination does not provide enough protection”, emphasize the authors of the article.

The researchers highlighted that the vaccine of inactivated virus are the best candidates for immunization in developing countries, since it's easier to transport and doesn't need ultracold chain storage. Over two billion doses of CoronaVac were already applied in 45 countries.

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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

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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.

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* 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

spike protein mutations T19R, Δ157-158, L452R, T478K, D614G, P681R, and D950N, the B.1.617.2 variant reproduces at a faster rate than previous lineages seen in China, posing substantial challenges for disease control (21, 22). This was the first outbreak of the B.1.617.2 variant in mainland China. It lasted from 21 May to 18 June 2021, during which time 167 persons infected with the Delta variant were identified in clinical settings, in quarantine, or through community screenings. In addition to case identification, contact tracing of the outbreak continued through 23 June 2021, after which no more cases were reported. Before the start of this outbreak, China had already started to rapidly roll out mass immunization campaigns, and Guangdong province was one of the forerunners of vaccine deployment. Specifically, more than 90 million doses of inactivated vaccine were administered in Guangdong before mid-June 2021. As such, the outbreak was an opportunity to gain insight into the effectiveness of inactivated vaccines against the B.1.617.2 variant.

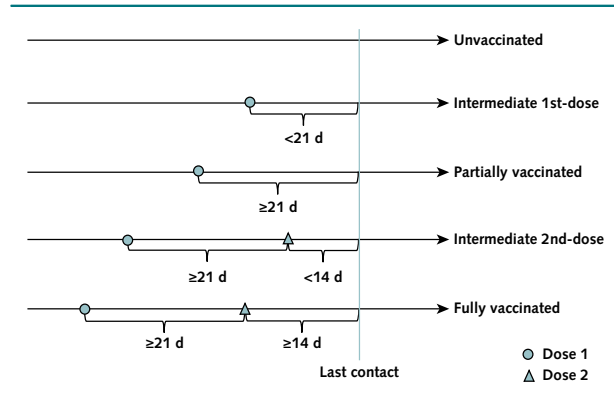
By analyzing vaccination, surveillance, screening, tracing, and quarantine data on China's COVID-19 prevention and control, we could assess the real-world effectiveness of inactivated vaccines against COVID-19 caused by the B.1.617.2 variant. More than 2 billion doses of inactivated COVID-19 vaccine have been administered in more than 80 countries and regions. Thus, evidence on the effectiveness of inactivated vaccines against the rampantly growing variant is critical for public health agencies and communities globally.

METHODS

Study Population and Design

The local outbreak in Guangdong was started by an imported infection from abroad; that patient transmitted it to a local resident, who was the index case patient. All secondary local cases were well traced and linked to the index case in a single long chain of transmission (23, 24). In accordance with national and provincial protocols for COVID-19 prevention and control, close contacts were defined as all people 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 (25). All close contacts were traced, mandatorily quarantined in centralized managed facilities, and followed with multiple reverse transcriptase polymerase chain reaction tests; they became part of our study cohort as the outbreak was proceeding and being managed. The Close Contacts Management section of the **Appendix** (available at [Annals.org](https://www.annals.org)) gives additional information on close contact definition and management, and the Laboratory Confirmation section gives information on specifications of test kits. Of note, all case patients were themselves close contacts of their upstream cases before they became infected. Therefore, the case patients and their close contacts made up a cohort together and should not be considered independent groups in an outbreak with a clear chain of transmission. We did a retrospective cohort analysis of all infected

Figure 1. Vaccination status definitions.



individuals and their close contacts identified in the Guangdong outbreak.

In addition to the index case (the first local infection), health authorities identified 12 500 individuals, including secondary case patients and close contacts. All positive specimens were subject to whole-genome sequencing. Individuals were excluded if basic demographic information was missing or if they received noninactivated vaccines. Because immunization campaigns in China requested a 21-day interval after the first dose and COVID-19 vaccines were provided only to adults until July 2021, persons who received 2 doses of vaccine but less than 21 days apart or were younger than 18 years were also excluded.

This study was approved by the institutional ethics committee of the Guangdong Provincial Center for Disease Control and Prevention. The data in the study were collected per administrative requirements of disease control and surveillance and were anonymized for analysis. Participants were informed about the requirements of disease surveillance and provided oral consent.

Vaccination Status

To determine vaccination status, we used the number of doses received and time elapsed since the most recent dose. On the basis of vaccination electronic records, participants were categorized into an unvaccinated group, a partially vaccinated (1-dose) group, and a fully vaccinated (2-dose) group. The unvaccinated group consisted of persons who did not receive any COVID-19 vaccines before their last known contact with a confirmed case patient. The partially vaccinated group comprised those who received their first dose 21 days or more 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 primary analysis was a 3-group comparison. Those who received their first dose within 21 days (intermediate first dose) or their second dose within 14 days (intermediate second dose) before the last known contact were excluded from the primary analysis to avoid ambiguity in definition. **Figure 1** illustrates categorization of the groups.

Outcomes

The primary outcome was pneumonia caused by the B.1.617.2 variant of SARS-CoV-2. Secondary outcomes were infections, symptomatic infections, and severe or critical illness associated with the B.1.617.2 variant. However, results are reported following the hierarchy of outcome severity. Symptoms and severity were defined according to China's Diagnosis and Treatment Protocol for COVID-19 Patients (26). Pneumonia was diagnosed using chest imaging characteristics. Severe cases were those in which the patient had a respiratory rate above 30 breaths/min, resting blood oxygen saturation of 93% or lower, or PaO₂-Flo₂ ratio of 300 mm Hg or lower (26). In critical cases, patients had respiratory failure leading to mechanical ventilation, experienced shock, or sustained any other organ failure that required intensive care (26). Severity was based on a participant's most serious manifestations during the follow-up period.

Characteristics and Covariates

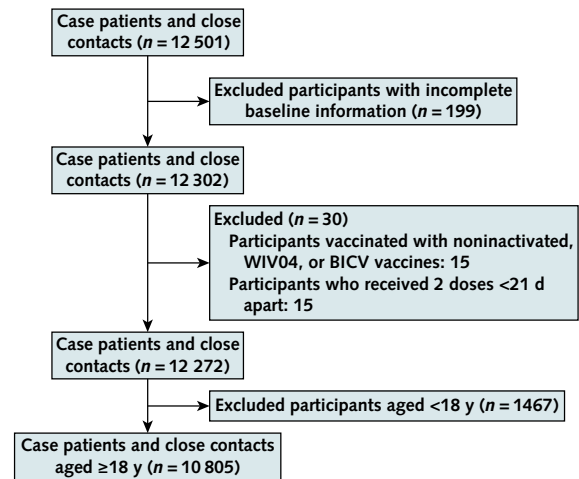
Epidemiologic investigators collected sociodemographic information, including age, sex, address, occupation, and contact frequency. These variables could potentially confound the vaccine effectiveness (VE) estimates by correlating with both vaccination and outcomes and were used as covariates in subsequent analyses. Age was categorized as 18 to 34 years, 35 to 49 years, or 50 years or older. In adherence to the national prevention and control scheme, investigators adjudicated contact frequency as occasionally, sometimes, or frequently. Contact frequency might correlate with vaccination status because vaccinated persons could be tempted to reduce adherence to nonpharmaceutical measures, such as social distancing (27, 28). Occupation might have been associated with vaccination status, in that professionals in occupations with high exposure risk were granted priority for vaccination during early 2021. To control potential confounding due to cross-occupation heterogeneity in the chances of vaccination and exposure to the virus during social interaction, we created indicators of working in restaurant services, working as a health care provider, and being currently unemployed. In addition, geographic area might lead to bias in estimation of VE if left unadjusted for because areas with different intensity of transmission might also have had different access to vaccines. Specifically, 2 subdistricts in Guangzhou (subdistricts A and B for simplicity) were epicenters of the outbreak. The cases in these 2 communities accounted for more than 60% of all outbreak cases. As such, residents of these 2 subdistricts could have had higher risk for exposure, yet access to vaccines in these communities was not necessarily the same as in other places. Therefore, an indicator was created for each of the 2 epicenter subdistricts and used as a covariate in addition to the sociodemographic variables.

Statistical Analysis

Primary Analysis

Characteristics of participants in each group were described using mean values (with SDs) and percentages. To estimate the unadjusted VE, the risk ratio (RR) of each

Figure 2. Study flow diagram.



outcome was calculated in reference to the unvaccinated group and subtracted from 1. In addition, we used multivariable logistic regressions to account for covariates that could potentially confound effect estimates. To estimate adjusted VE (aVE) from multivariable logistic regressions, we first calculated the adjusted RR (aRR) that equaled the ratio of the predicted event probability in each vaccination group to that in the unvaccinated group; the Adjusted Risk Ratio section of the Appendix elaborates on this (29–31). The aVE was then calculated as $1 - aRR$. We used aRRs to calculate aVEs because RRs are intuitively understandable for cohort studies and because odds ratios consistently underestimated RRs for protection effects, leading to potentially exaggerated VE estimates (32). The SEs of aRRs were estimated using the delta method, which is frequently used for nonlinear transformations of regression coefficients (33). We used Stata, version 16 (StataCorp), with the logit routine and its postestimation features for analyses.

Sensitivity Analysis

In a prespecified sensitivity analysis, vaccination status was based on each person's number of doses before the outbreak. In this analysis, anyone who received their first dose but not their second dose before 7 May 2021 (14 days before 21 May 2021) was assigned to the partially vaccinated group, whereas those who received both doses before 7 May 2021 made up the fully vaccinated group. Those who received the initial dose after 7 May 2021 were excluded from this analysis. In addition, a between-dose window was not considered when determining vaccination status.

We also did several post hoc sensitivity analyses, making 1 change to the base case at a time (Post Hoc Sensitivity Analyses section of the Appendix). Specifically, we included all vaccination statuses as distinct exposure groups, used cluster-robust SEs, and replaced logistic regressions with Poisson regressions that allowed direct estimation of incidence rate ratios in the sensitivity analyses. To examine the

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](https://www.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).

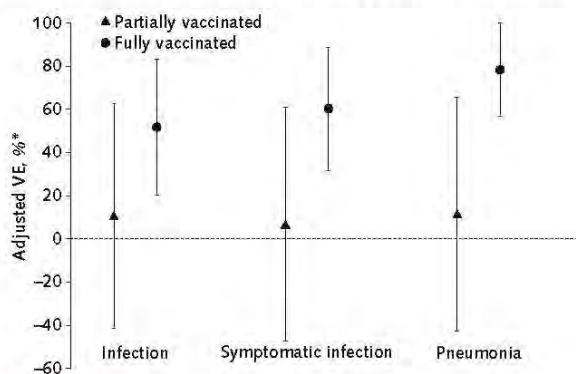
CoronaVac and HB02 are both authorized by the World Health Organization for emergency use and together accounted for almost half of the COVID-19 vaccine doses dispensed globally as of October 2021 (37, 38). Therefore, our study has important policy implications. First, it is critically important to continue 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-world 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.

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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:

$$P_{FV} = \frac{1}{N} \sum_{i=1}^N \Pr(y_i = 1 | X, \text{full vaccination} = 1),$$

$$P_{NV} = \frac{1}{N} \sum_{i=1}^N \Pr(y_i = 1 | X, \text{no vaccination} = 1).$$

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. Three doses of CoronaVac induce antibodies against omicron in 95% of vaccinees, Chinese study shows

In a paper published in the Nature journal, researchers from the Chinese Academy of Sciences have shown that a booster dose of CoronaVac promotes immune response against the omicron variant of SARS-CoV-2 in 95 percent of those vaccinated, and increases the capacity to neutralize this strain by rapidly activating memory B cells, which produce antibodies.

Chinese scientists collected blood samples from 60 volunteers who received three doses of CoronaVac to assess neutralizing antibody titers against the omicron and delta variants - live virus was used in this study. None of the recruited individuals had been infected with the SARS-CoV-2 virus prior to the analysis.

According to the research, after the third dose, 95% of the participants showed seroconversion against omicron. The neutralizing antibody titers against the original strain (from Wuhan, which triggered the pandemic) and against the delta and omicron variants were 254, 78 and 15.5, respectively. The antibody titers count, however, represents only one part of the immune response, which is completed by

memory B cells, which can recognize an invader, divide, and quickly start producing antibodies to fight it.

To assess the potential of the immune memory of the three-dose vaccinees, the scientists isolated 323 B-cell-derived monoclonal antibodies, half of which (163) recognized the receptor-binding domain (RBD) of the virus. A subset of the monoclonal antibodies (24 of 163) that was also identified was able to neutralize all variants of concern of SARS-CoV-2, including omicron.

According to the researchers, studies have shown that omicron can resist the antibodies produced with two doses of vaccine, which reinforces the need for a third dose. "Our study revealed that the three-dose CoronaVac vaccination regimen induces an enhanced immune response, with significantly increased neutralization. In addition, a subset of highly potent neutralizing antibodies against the variants of concern was present in at least four individuals [among 60 investigated]."

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Memory B cell repertoire from triple vaccinees against diverse SARS-CoV-2 variants

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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

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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 vaccines³⁻⁵, 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 world⁶. 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 Δ 69-70 in Alpha, N501Y in Alpha, Beta and Gamma, P681H in Alpha and Delta, are presumably associated with enhanced transmissibility, while many substitutions, including G142D/ Δ 143-145, 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

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 vaccine¹². 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 NT₅₀) 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 NT₅₀ 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, NT₅₀ titer against Delta was 6.6 with a 5.1-fold reduction when compared to WT, but none of the serum specimens had an NT₅₀ 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

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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 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$, 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 $\mu\text{g/ml}$ for well-studied therapeutic antibodies like VIR-7831 and DXP-604,

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 ($IC_{50} < 0.2 \mu\text{g/ml}$) 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 individuals⁵, 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 structures⁷, 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 $\mu\text{g/ml}$, respectively), one mAb (XGv282 with IC_{50} of 0.268 $\mu\text{g/ml}$) with median neutralizing activities against Omicron, but high neutralizing potency against other four VOCs, and one mAb (XGv265 with IC_{50} of 7.479 $\mu\text{g/ml}$) 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

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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 variants²², 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 $\sim 10^\circ$ 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 orientations²⁰, but in a manner similar to those observed for DH1047, BD-812 and REGN10987; antibodies known to generally neutralize most VOCs with high potency²⁵⁻²⁷, 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

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

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 variant²⁸. 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

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,

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AZD8895 and AZD1061². 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 ($IC_{50} < 0.2 \mu\text{g/ml}$) 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 microenvironments 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-tuned 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 flu³³. 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.

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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

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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 described¹⁴ 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 °C for 30 min for inactivation. The heat-treated samples or monoclonal antibodies (mAbs) were subject to serial dilution from 1: 4 or 50 µg/ml with DMEM in two-fold steps and mixed with a virus suspension containing 100 TCID₅₀ at 36.5°C for 2h, after which, the mixtures were added to wells seeded with confluence Vero cells and incubated at 36.5°C for another 5 days in a humidified 5% CO₂ 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-Muench¹⁴.

Pseudovirus neutralization assay

The pseudotyped viruses bearing the S protein were generated, aliquoted and restored as previously described¹⁸. Briefly, 293T cells were first transfected with the plasmid embedded with the S gene of WT or VOC/VOI (Alpha, Beta, Gamma, Delta, Lambda 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 (IC₅₀).

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 addition 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% CO₂, rotating at 130 rpm. The cell supernatants were

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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⁺ 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 described³⁵.

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 previously⁷. Then the clones were transiently transfected into mammalian HEK293F cells and incubated for 5 days in a 5% CO₂ 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 previously³⁶. 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).

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 Analysis 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)

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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 Inc.), 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 $60 \text{ e}^- \text{ \AA}^{-2}$) 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 \AA .

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 package³⁷. 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 cryoSPARC³⁸, 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 previously³⁹. The resolution of each structure was determined on the basis of the gold-standard Fourier shell correlation (threshold = 0.143) and evaluated by

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 cryo-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,

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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 strain²⁸. 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 TCID₅₀ 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 previously¹⁷. 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 μ m 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

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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.

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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.

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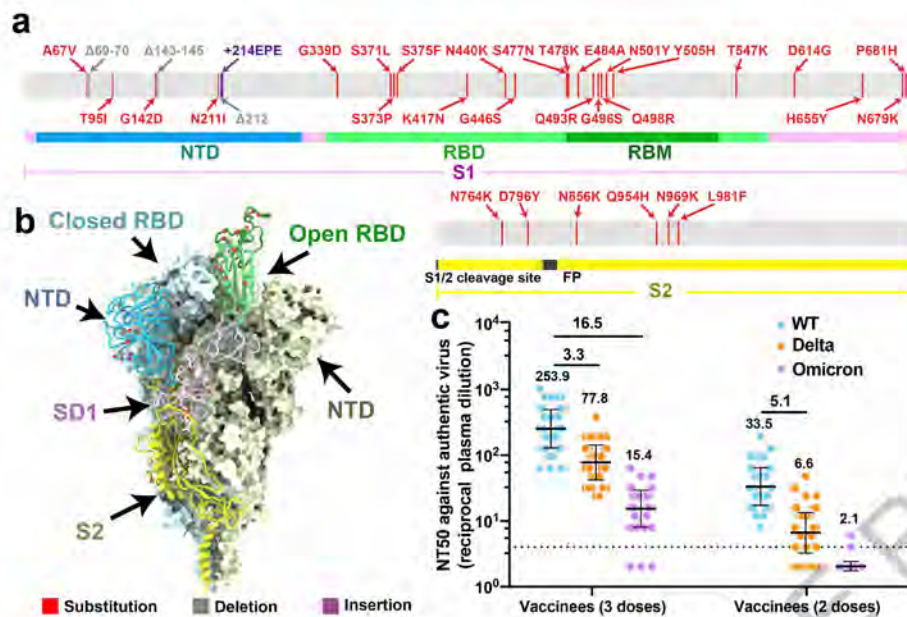


Fig. 1 Evolution and neutralization characteristics of Omicron variant. **a**, A linear representation of Omicron S with mutations marked on. The replacements are marked in red; deletions are in grey and insertions are in purple. **b**, Distribution of mutations of Omicron on the cryo-EM structure of 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 NT₅₀ ± SD of technical triplicates. The dotted line represents the detection limit. NT₅₀ 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.

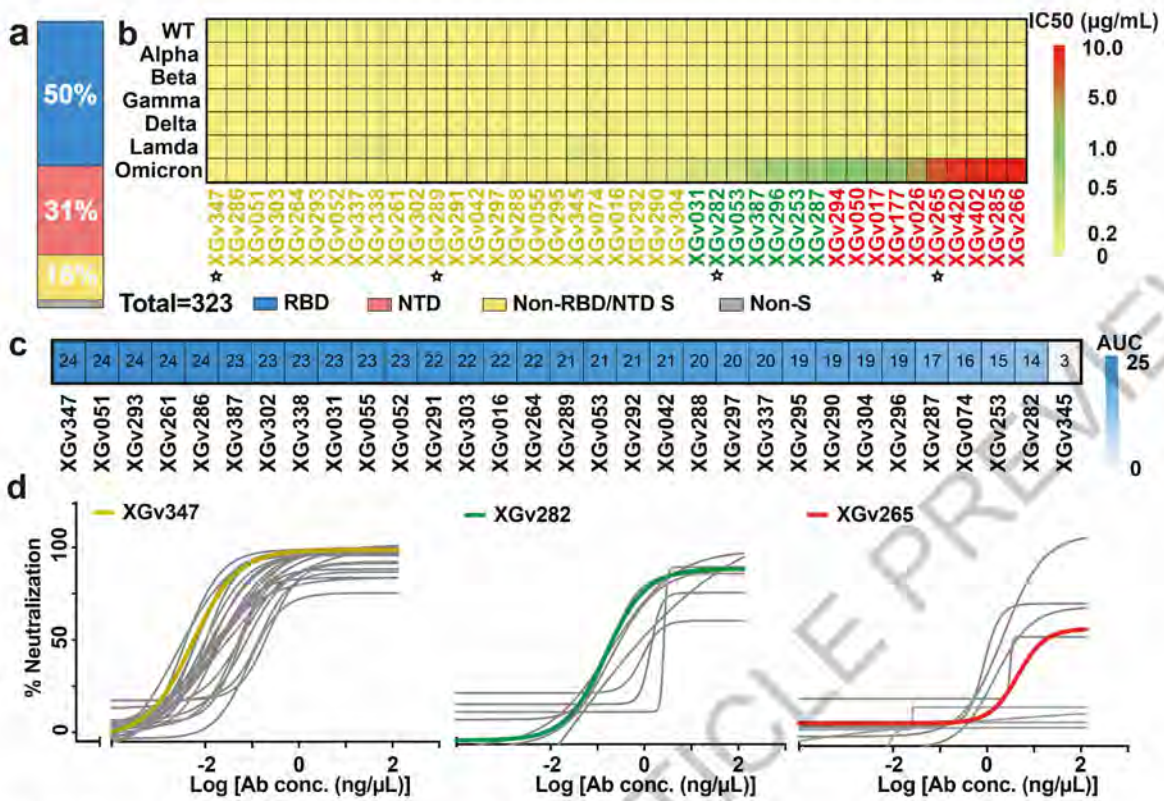


Fig. 2 Characteristics of a subset of broadly neutralizing antibodies from recipients of a booster immunization. **a**, Vertical slices chart shows the gross binding epitope distribution of mAbs isolated from the individuals who received three doses of inactivated vaccines. Total number of antibodies 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 $\mu\text{g}/\text{ml}$; green: 0.020-1.000 $\mu\text{g}/\text{ml}$; red: 1.000-10.000 $\mu\text{g}/\text{ml}$). 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.

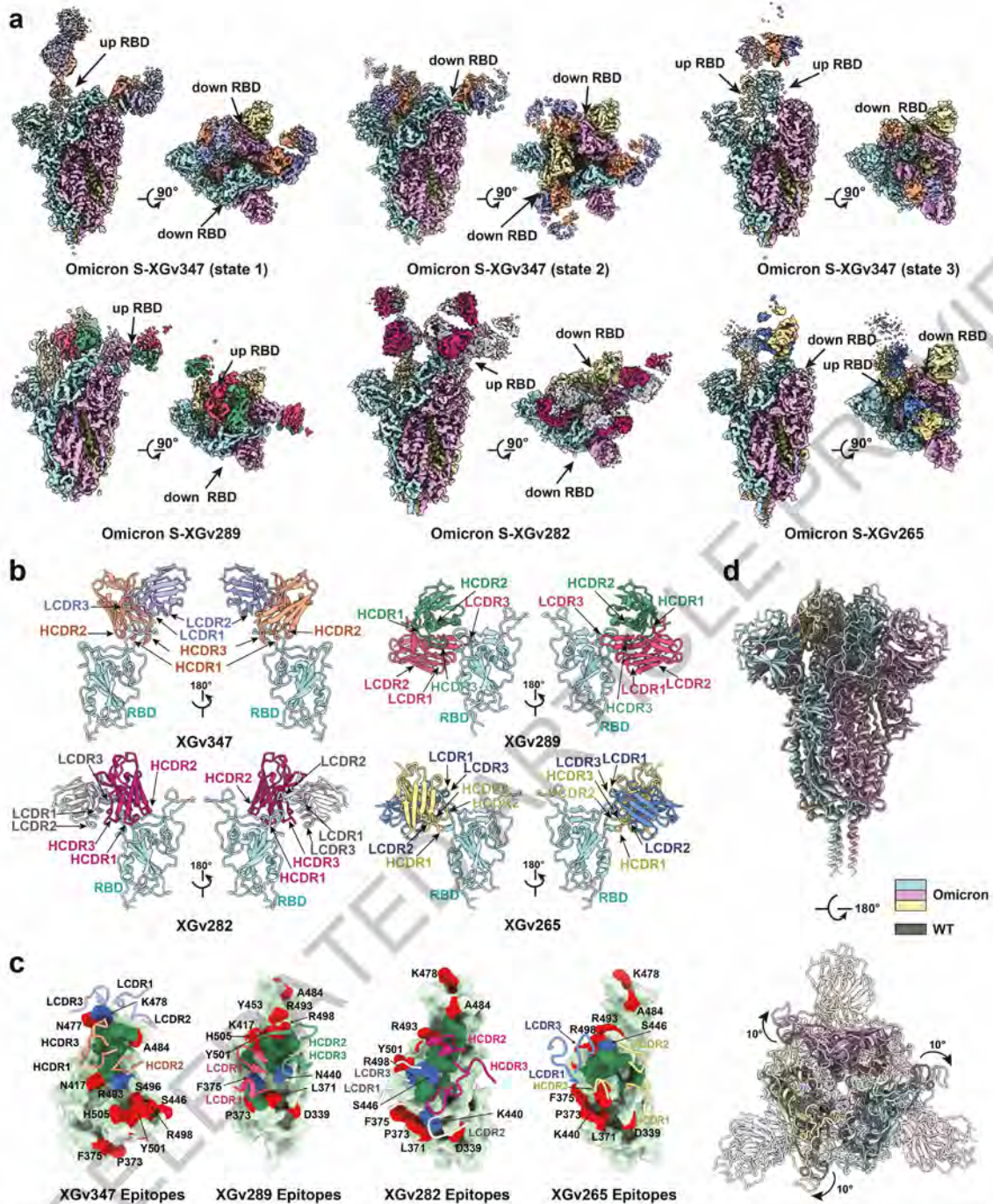


Fig. 3 Structural basis of the broad and potent neutralization of representative antibodies. **a.** Side view and top view of Cryo-EM maps of SARS-CoV-2 Omicron S trimer in complex with XGv347 (state 1-3), XGv289, XGv282 and XGv265. For XGv347-S-complex, state 1, one up RBD and one down RBD; state 2, 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.

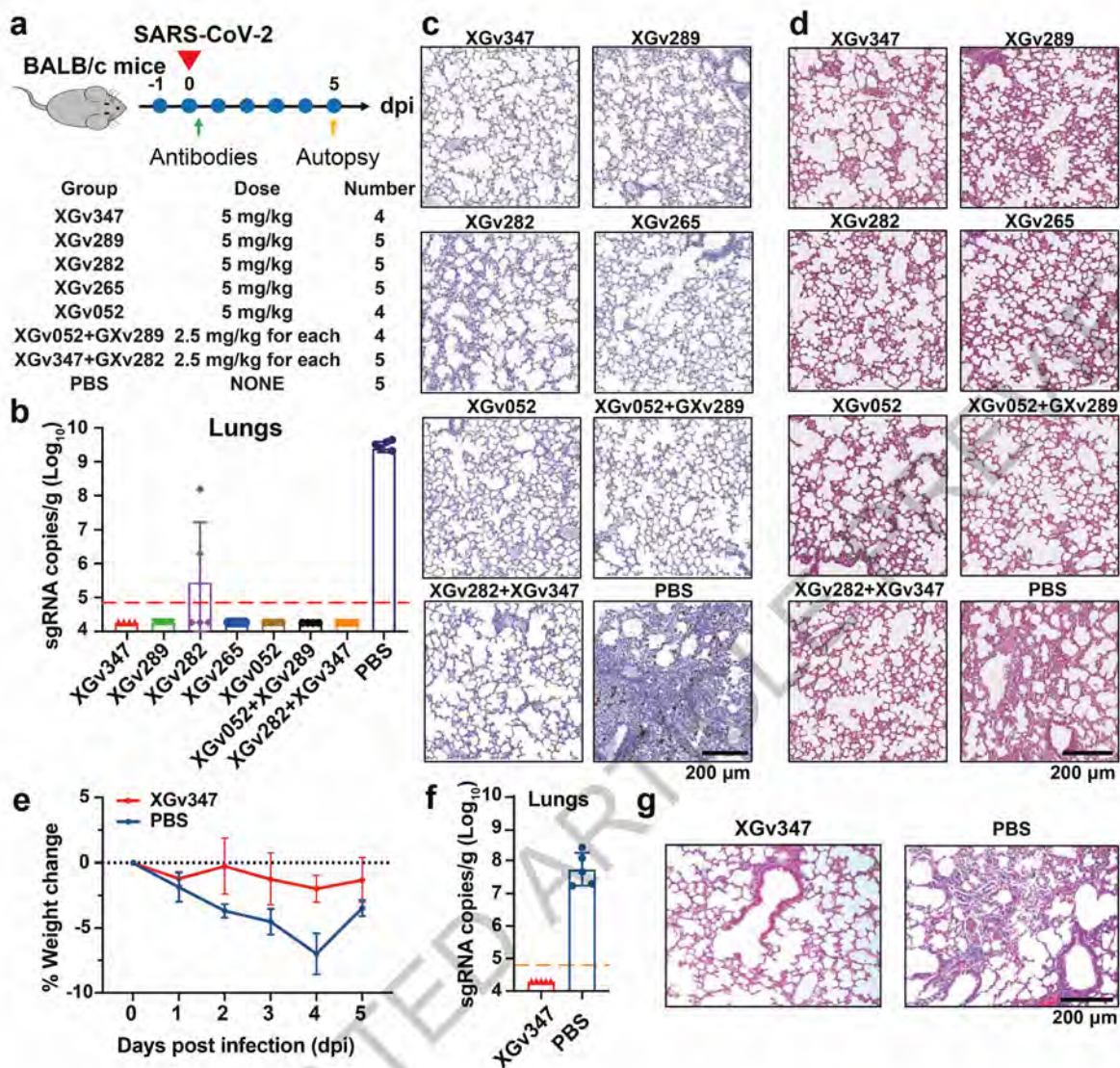
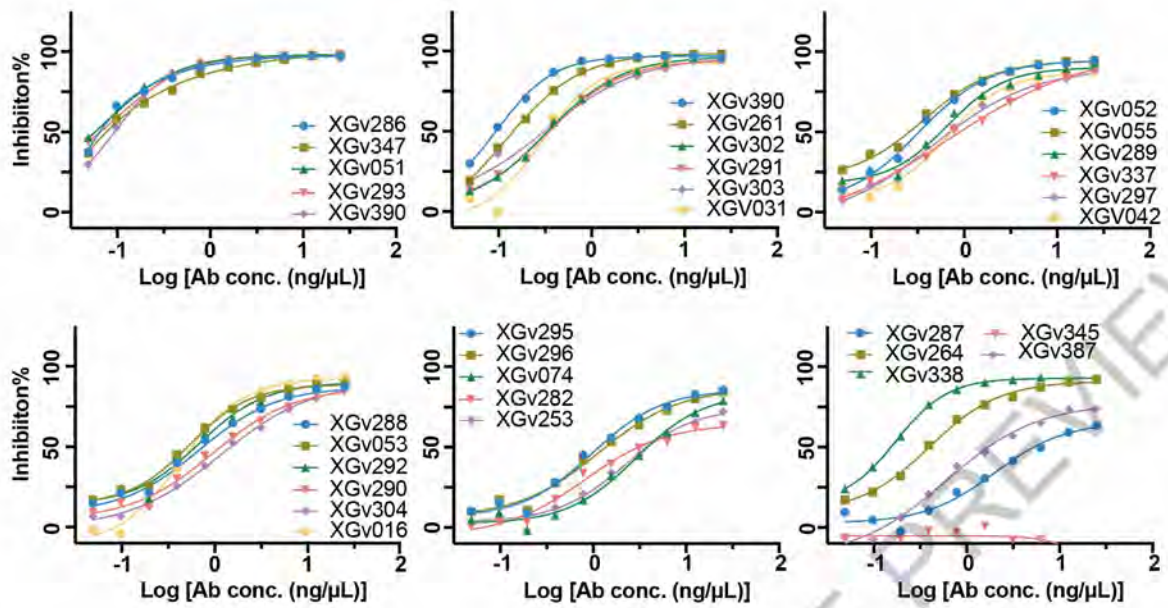
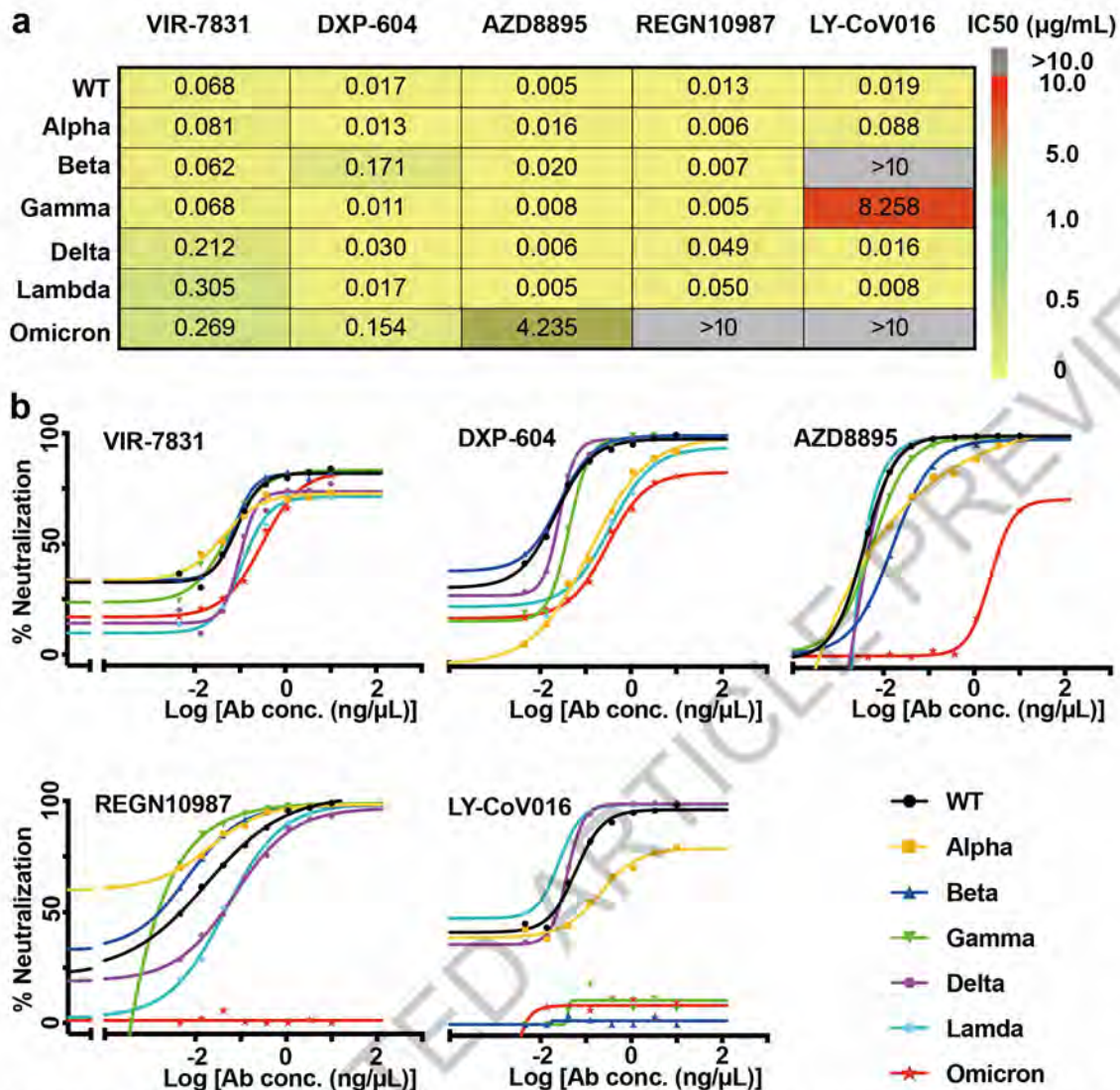


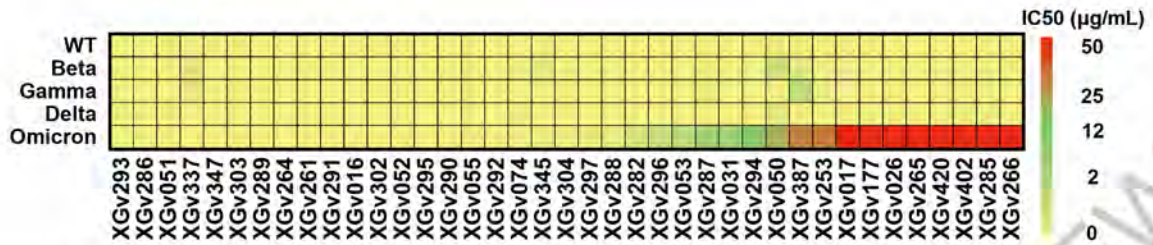
Fig. 4 Protection against SARS-CoV-2 Beta and Omicron variants challenge in mice. **a**, Experimental design for protection assay against Beta variant challenge. $n = 4$ mice in XGv347, XGv052, and XGv052 + XGv289 groups; $n = 5$ mice in other groups. **b to d**, Examination of lung tissues of Beta variant 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 \pm 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 μ m. **d**, Histopathological analysis of lung samples at 5 dpi. Scale bar: 200 μ m. **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 \pm SD. Dashed line represents limit of detection. **g**, Histopathological analysis of lung tissues from both two groups. Scale bar, 200 μ m. 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.



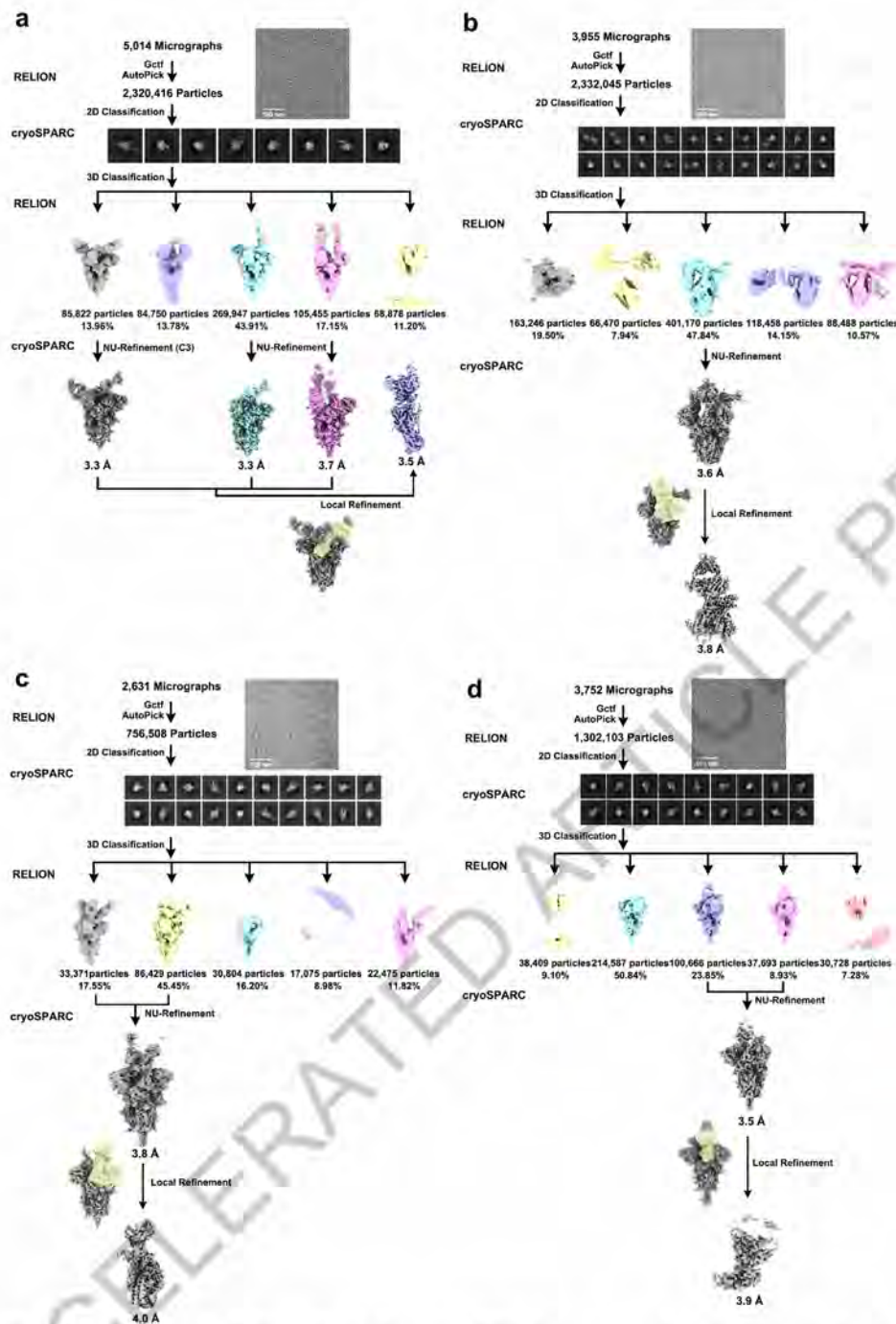
Extended Data Fig. 2 Characteristics of representative antibodies against pseudotyped viruses. **a**, Heatmap representation of five therapeutic mAbs approved or in clinical trials against pseudotyped viruses with the S proteins of wild-type or variants of concern or interest (Alpha, Beta, Gamma, Delta, Lambda and Omicron). **b**, Neutralization curves for these mAbs in correspondence with **a**. Mean of two experiments is shown.



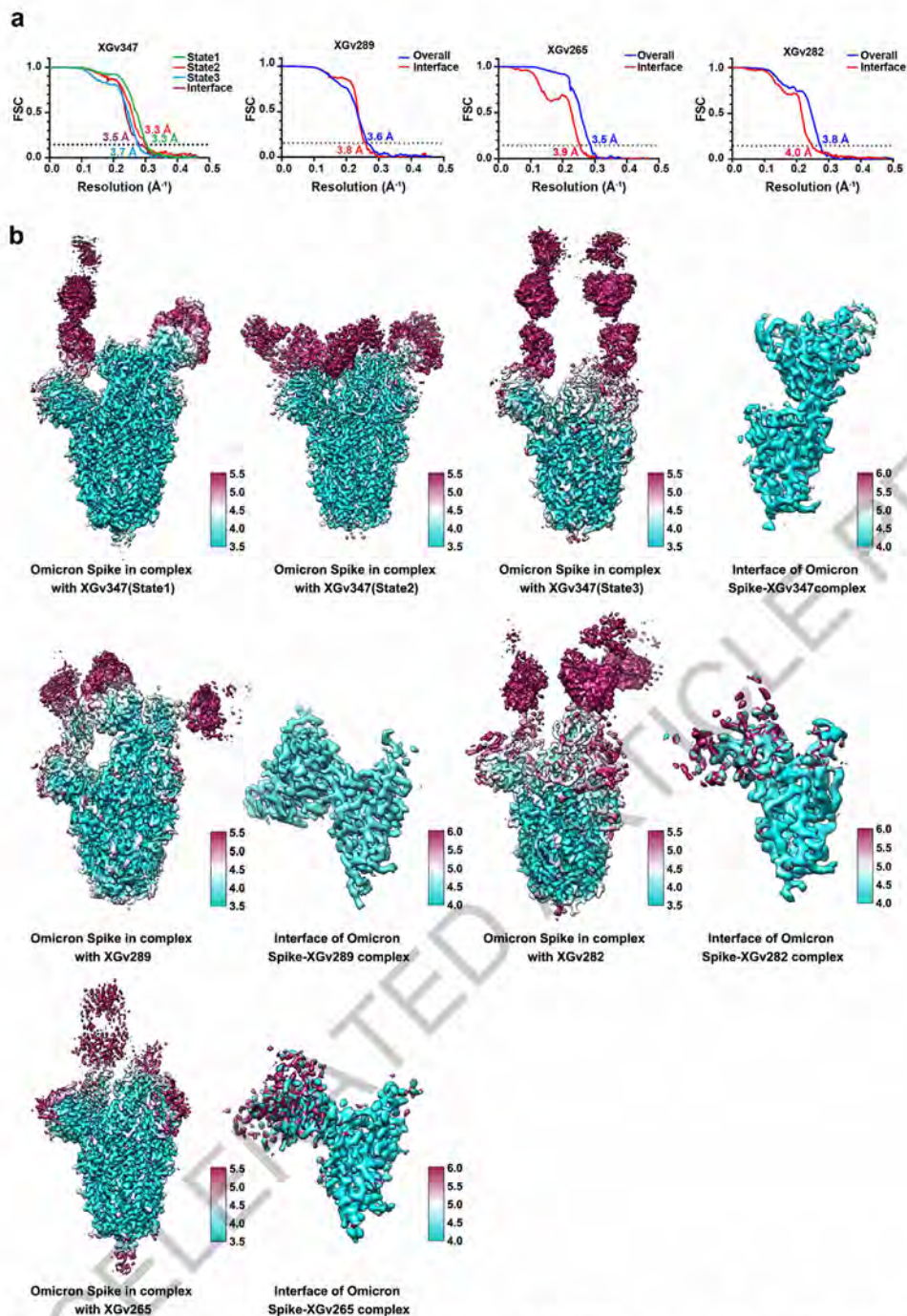
Extended Data Fig. 3 Heatmap representation of representative mAbs against WT and variants of concern. Color bar on the right showed the gradient of IC50 of different antibodies against the authentic WT and variants of concern. All experiments were performed in duplicate.

	Class I			Class II			Class III		Class IV			Class V			Class VI			NC	
	XGv013	XGv026	P4A1	XG017	414	H4	XGv013	917	XGv016	S309	XG014	XGv020	XG025	FC08	XGv009	XG011	A34-2		Fe05
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	89
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	63	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	63	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	63	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	3	63	79	73	84	3	6	93	86	4	5	4	86	72	79	82	84	134	86
XGv265	3	75	77	62	83	3	6	87	86	16	7	5	74	64	80	74	76	108	89
XGv266	3	73	78	87	87	3	5	91	80	12	6	4	85	64	83	86	76	127	86
XGv282	3	81	79	105	77	3	5	83	10	5	43	4	93	76	11	70	89	106	88
XGv289	3	57	78	74	72	3	7	91	77	8	71	5	87	72	82	67	86	143	87
XGv296	3	51	74	64	67	3	6	86	87	5	79	5	80	64	85	67	72	122	87
XGv345	3	88	95	87	92	3	7	97	31	22	7	4	76	12	14	248	132	121	65

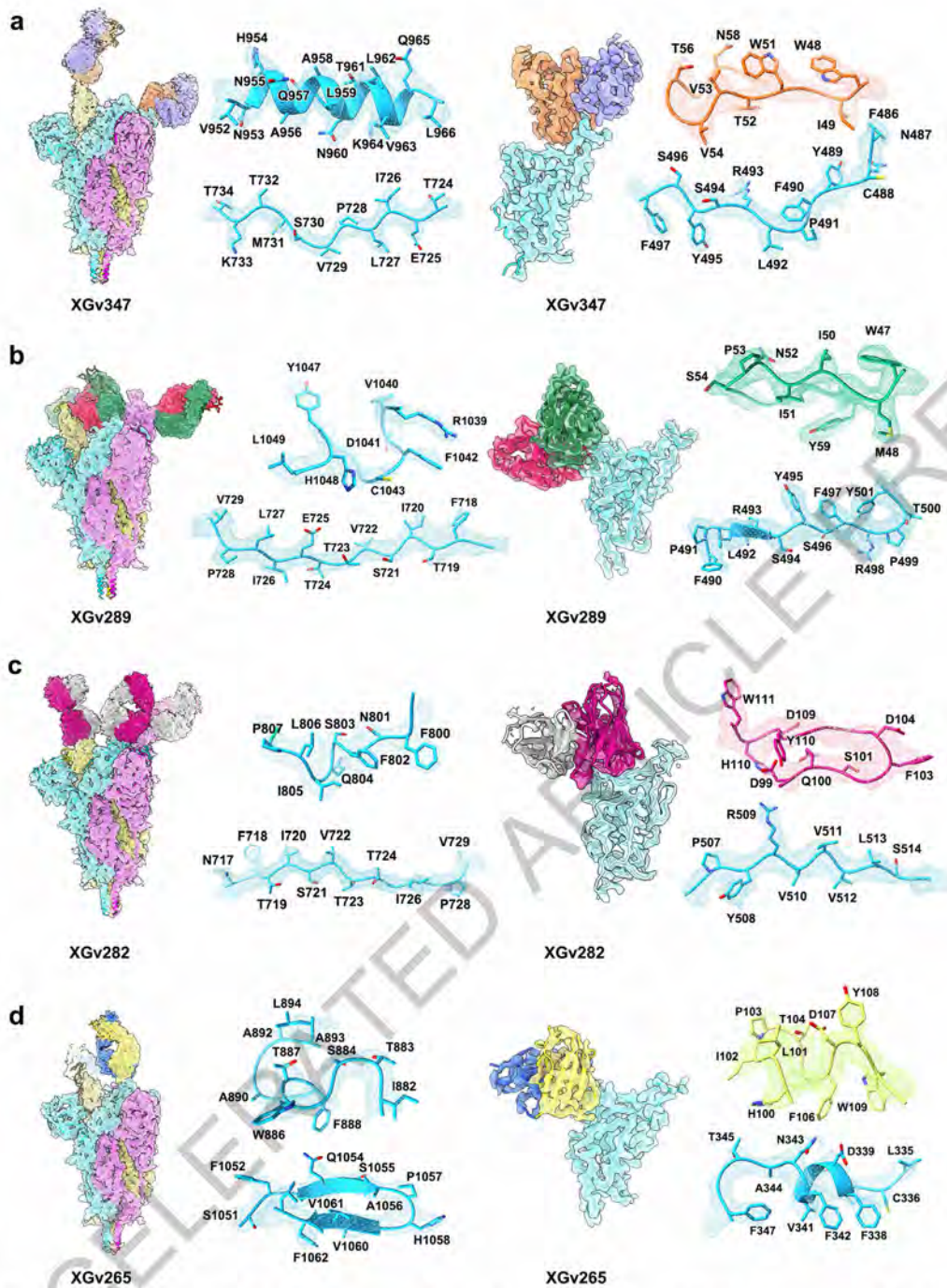
Extended Data Fig. 4 Data sheets of ELISA assay of representative mAbs against Omicron RBD. Different Classes of mAbs (Class I-VI) are colored by yellow, green, red, blue, brown and magenta, respectively. Values are filled with black (>75), grey (50-75), silver (25-50) and white (<25). Each data is the 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. Scale 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 atomic 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. 3a. Residues are shown as sticks with oxygen colored in red, nitrogen colored in blue and sulfurs colored in yellow.

Heavy Chain

	1	10	20	30	40	50	60
XGv347	QMQLVQSGPEVKKPGTSTVKVSKKASGFTTIDVSSLOWVROARGORLEWIGWIVIGTNTN						
COV2-2196	QMQLVQSGPEVKKPGTSTVKVSKKASGFTTMS.S.AVQWVROARGORLEWIGWIVIGSNTN						
A23-58.1	QMQLVQSGPEVKKPGTSTVKVSKKASGFTTSS.AVQWVROARGORLEWIGWIVIGSNTN						

	70	80	90	100	110	120
XGv347	YAPRFQERVITITDKSTSTAYMELSSLRSED TAVYYCAAPFCSEISCSDFDHWGGTKV					
COV2-2196	YAKRFQERVITITRDMSTSTAYMELSSLRSED TAVYYCAAPFCSSISCNDGFDHWGGTMV					
A23-58.1	YAKRFQERVITITRDMSTSTAYMELSSLRSED TAVYYCAAPFCSSNVVCYDGFHWGGTMV					

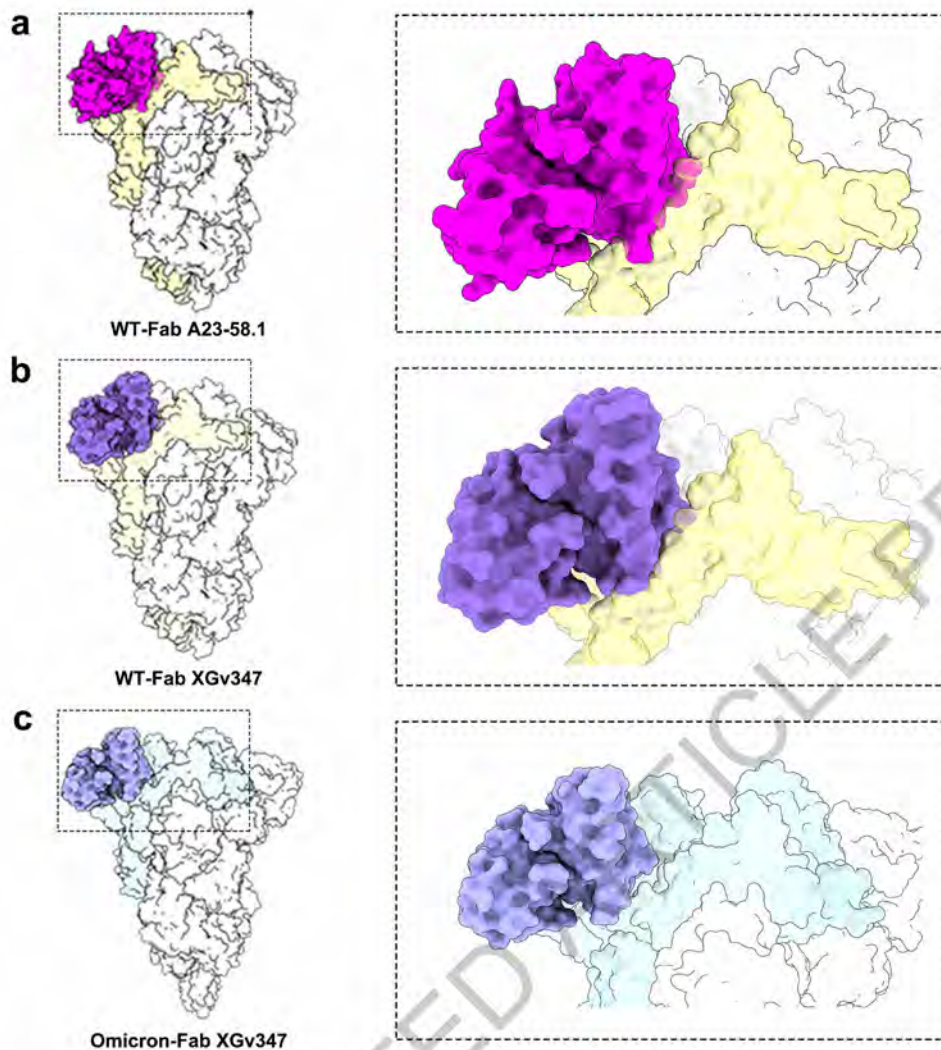
XGv347	TVS.
COV2-2196	TVS
A23-58.1	TVS

Light Chain

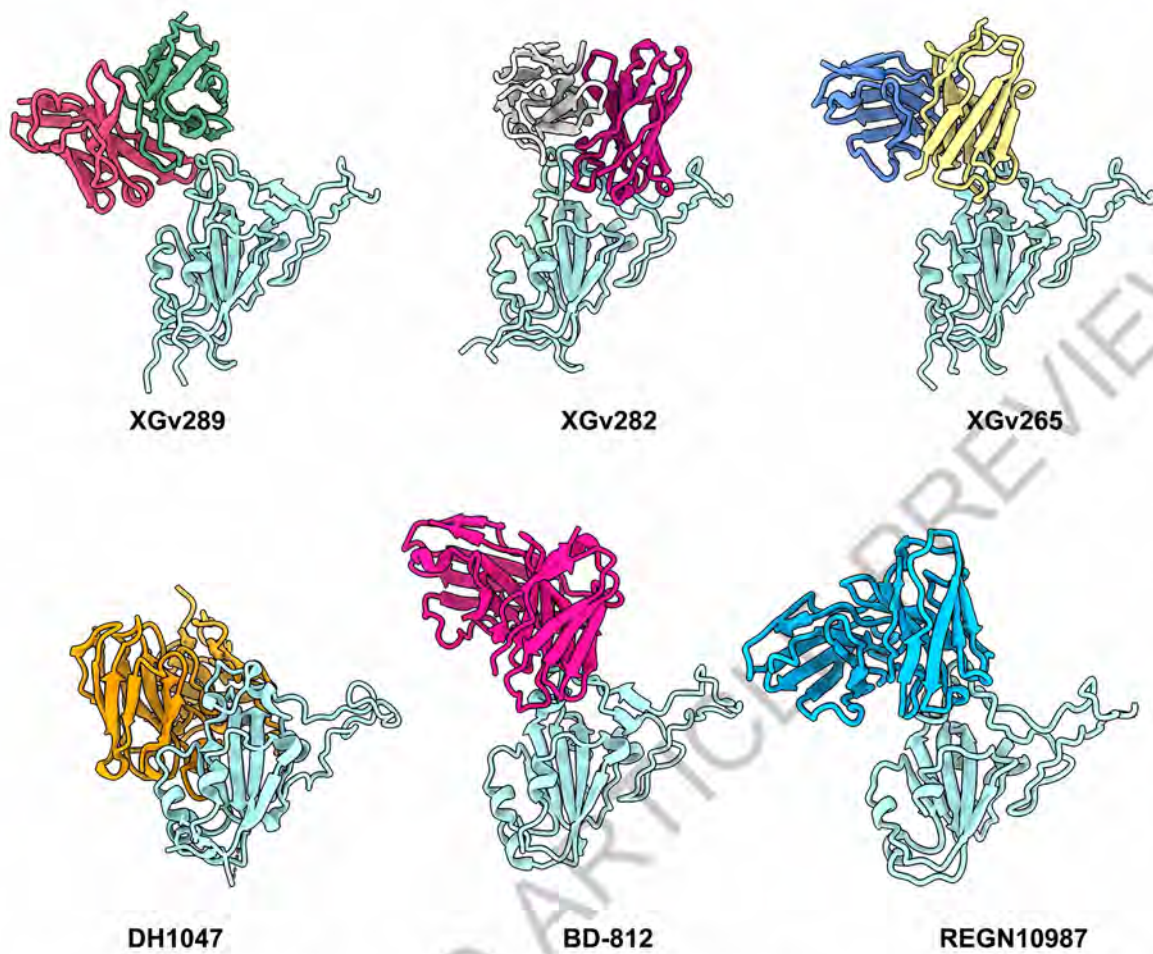
	1	10	20	30	40	50	60
XGv347	EIVLTQSPGTLISLSPGERATLSCRASQSVRIISYLAWYQOKPGQAPRLLISSGSSSRATGIP						
COV2-2196	EIVLTQSPGTLISLSPGERATLSCRASQSVSSSYLAWYQOKPGQAPRLLIYGASSSRATGIP						
A23-58.1	EIVLTQSPGTLISLSPGERATLSCRASQSVSSSYLAWYQOKPGQAPRLLIYSASSSRATGIP						

	70	80	90	100
XGv347	DRFSA SGSGTDFLTISRLEPEDFAVYVCOQYANSP.WTFGQGTKVEV.			
COV2-2196	DRFSG SGSGTDFLTISRLEPEDFAVYVCOHYGSSRGWTFGQGTKVEIK			
A23-58.1	DRFSG SGSGTDFLTISRLEPEDFAVYVCOQYGTSP.WTFGQGTKVEIK			

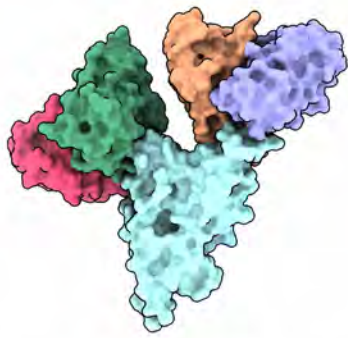
Extended Data Fig. 8 Multiple sequence alignment of XGv347, CoV2-2196 and A23-58.1 Multiple sequence alignments of heavy chains and light chains of XGv347, CoV2-2196 and A23-58.1 were performed, respectively. Paratopes of XGv347 binding to Omicron variant RBD are highlighted by green boxes.



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.



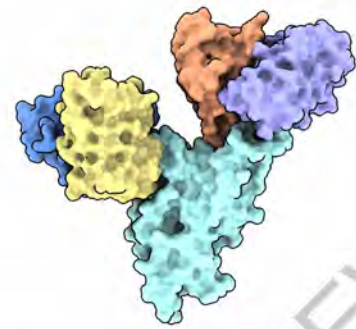
Extended Data Fig. 10 Binding modes of XGv289, 282 and 265. Binding modes of XGv289, XGv282 and XGv265. RBD is colored in light cyan and color scheme of XGv289, XGv282 and XGv265 is the same as in Fig. 3a. DH1047, BD-812 and REGN10987 are colored in orange, deep pink and blue, respectively.



XGv289-347



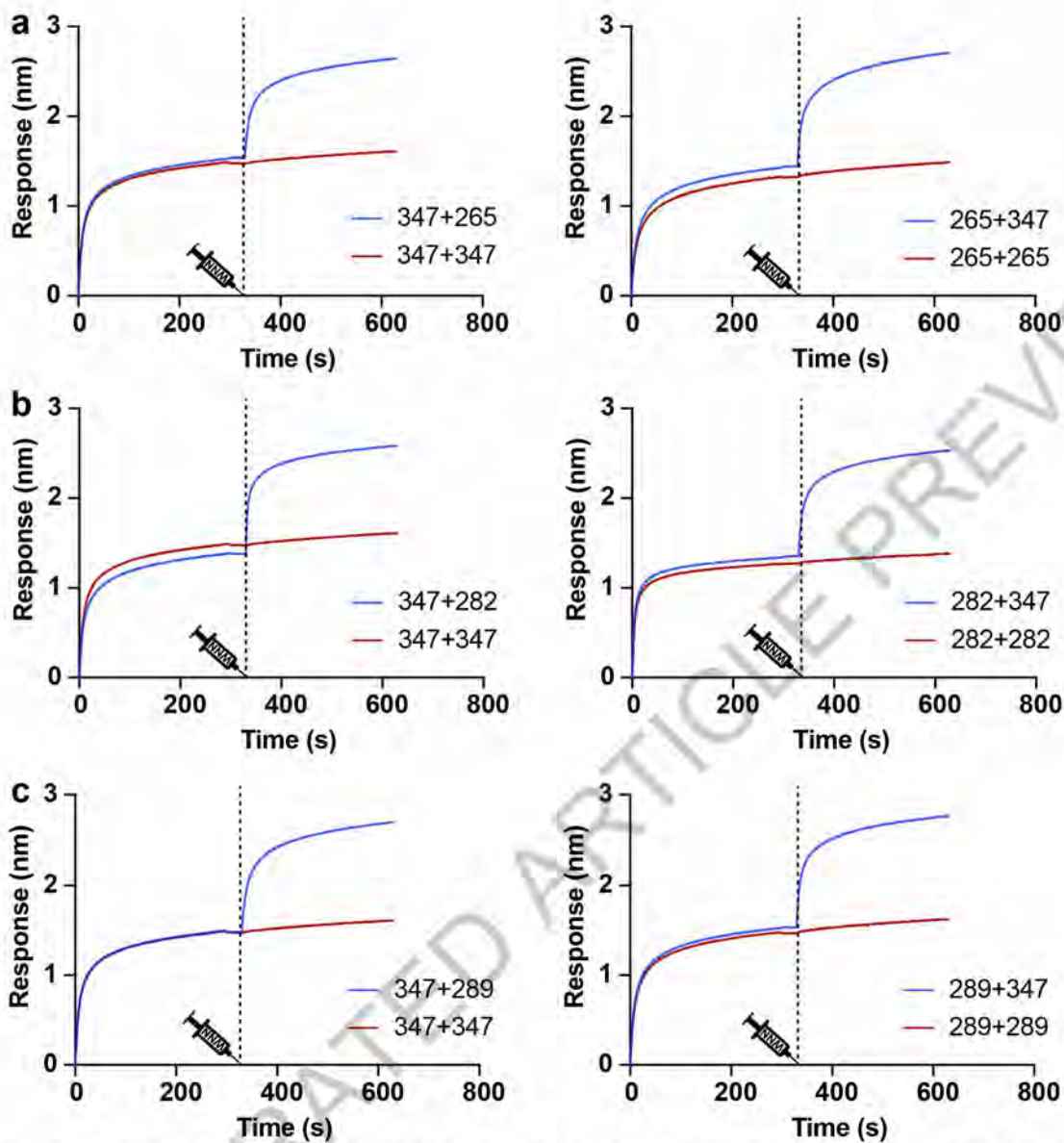
XGv282-347



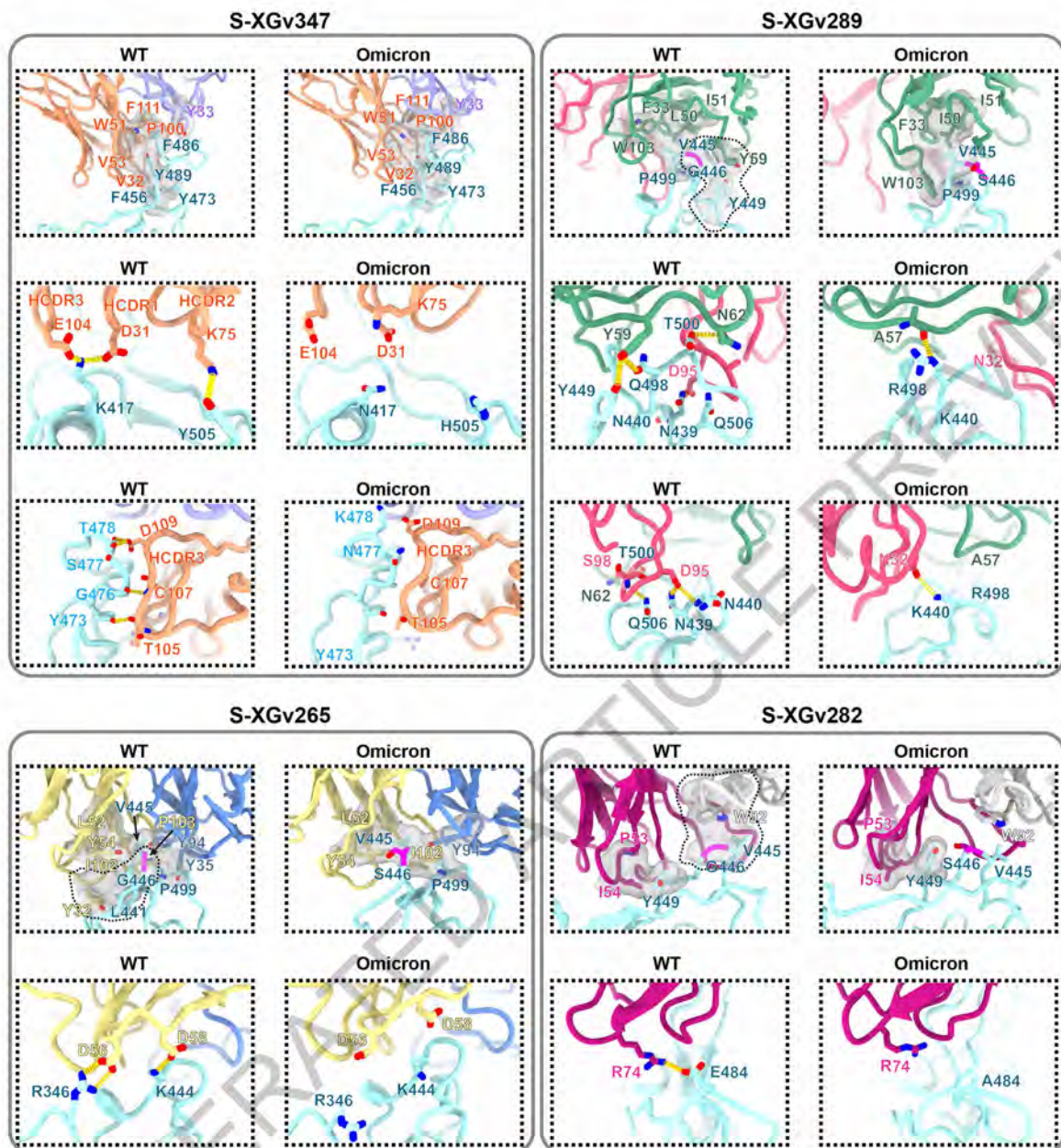
XGv265-347

Extended Data Fig. 11 Structural fitting. XGv265, XGv282 and XGv289 are superimposed onto XGv347 and all structure are shown as surface.

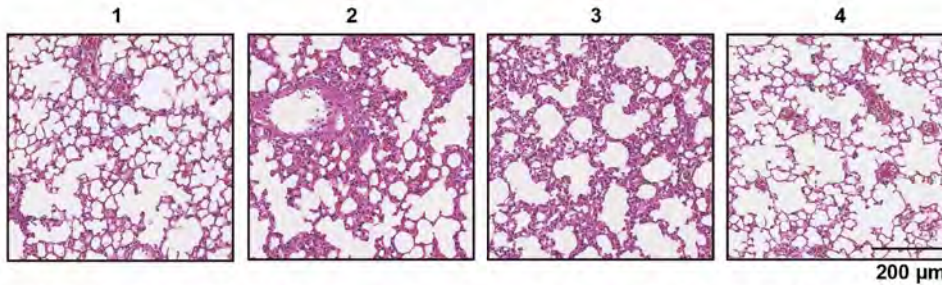
ACCELERATED ARTICLE PREVIEW



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.3a. 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.

ACCELERATED ARTICLE PREVIEW

	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 7WES	XGv347-RBD-interface with EMD-32447 PDB 7WED	XGv289-RBD-interface with EMD-32449 PDB 7WEF	XGv282-RBD-interface with EMD-32581 PDB 7WLC	XGv265-RBD-interface with 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-/Å ²)	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 β factor (Å ²)	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)	$\Delta\Delta G$ (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.

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- 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.

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
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Data availability The 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. Furthermore, the atomic coordinates of XGv265, XGv282 and XGv289 have been deposited in the protein data bank under accession code 7WEB, 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

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- 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](https://www.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

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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		Methods	
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<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms		
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants		
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data		
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern		

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

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

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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.

2.5. Booster dose of CoronaVac can neutralize variants of concern, study shows

A research published in *Emerging Microbes & Infections* journal demonstrated that the third dose of CoronaVac protects not only against the original strain of SARS-CoV-2, but also against the variants alfa, beta and delta. Besides, the memory of the T cells may be awakened quickly after the booster dose, if the person gets infected by the virus. Published in November of 2021, the study was conducted by Chinese scientists of the Biomedicine Institute from the Chinese Academy of Medical Sciences.

The researchers evaluated the capacity of protection against the variants alfa, beta and delta in blood samples of 53 patients vaccinated with CoronaVac and of 12 animal models, 14 days after the booster dose - administered 8 months after the second dose.

The seroconversion (antibodies production) exceeded 90% and the antibodies were capable of neutralizing the variants.

In a previous analysis, scientists collected samples of six of the 53

volunteers to detect IgG antibodies, neutralizing antibodies and memory T cells response against the original strain of the coronavirus.

The IgG antibodies and the neutralizing antibodies gradually increased after five days and the seroconversion reached 100% in 14 days. The response of the T cells was also fast.

“Our findings indicate that, although the neutralizing antibodies decreased with time after both doses, the response of antibodies can be quickly awakened with the third dose, and the immunological memory of the T cells is still active”, informed the authors.

The scientists added that it is essential to keep analyzing the persistence of the immunity and the effectiveness of the booster dose of the vaccines, conducting long term clinical trials.

Published on: 11/16/2021



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](https://doi.org/10.1080/22221751.2021.1996210)

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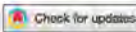


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LETTER

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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

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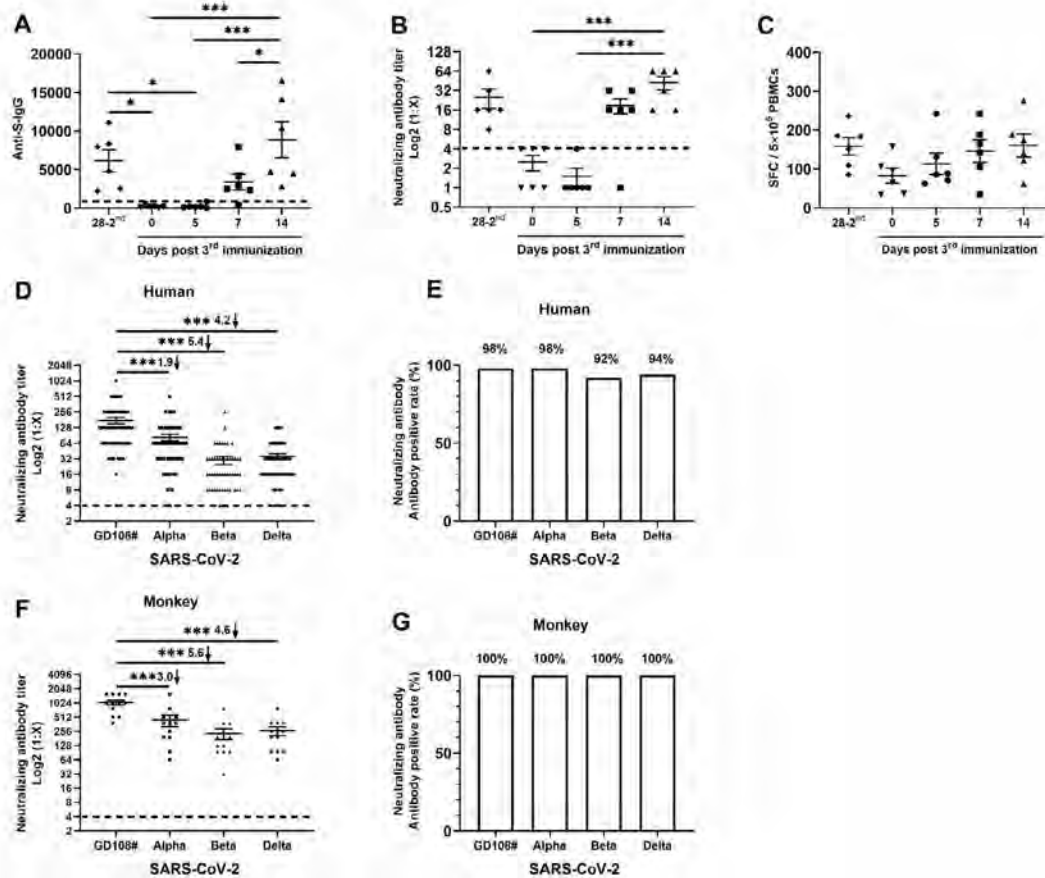


Figure 1. Antibody response elicited by a third booster dose of inactivated SARS-CoV-2 vaccine can neutralize SARS-CoV-2 variants of concern. (A–C) Enzyme-linked immunosorbent assay (ELISA) antibody against S protein, neutralizing antibody against original strain (GD108#), and the IFN- γ -specific T-cell 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.

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CoronaVac

What science proves

2.6. CoronaVac is more than 75% effective against alpha, gamma and delta variants; only 2% of Chileans vaccinated in phase 3 developed Covid-19

Two studies published by Chilean scientists provide evidence that CoronaVac, a vaccine from Butantan and the Chinese pharmaceutical company Sinovac, is efficient in combating Covid-19 and effective against the new variants of SARS-CoV-2. In the first study, the indicators of neutralizing antibodies generated by the vaccine were above 97% against the original strain of the virus, above 80% against the alpha and gamma variants, and above 75% against the delta variant. In the second study, the efficacy of CoronaVac in preventing Covid-19 cases was over 90% in a group of over two thousand people.

Both studies were conducted by scientists from the Pontificia Universidad Católica de Chile, Instituto de Salud Pública de Chile and the Universidad de Chile, and were published in the scientific journal *Frontiers of Immunology*. The importance of the studies is due to the fact that the vaccination in the Andean country was predominantly done with CoronaVac, with 70% of the people receiving the Butantan immunizer.

CoronaVac efficacy against SARS-CoV-2 variants

According to the study “Recognition of variants of concern by antibodies and T cells induced by a SARS-CoV-2 inactivated vaccine”, CoronaVac promoted antibodies capable of blocking the receptor-binding domain (RBD, specific parts of the coronavirus that allow it to invade and infect human cells) of all SARS-CoV-2 variants of concern. The neutralizing antibody seropositivity rates were over 97% for the original strain, over 80% for the alpha and gamma variants, over 75% for the delta variant, and over 60% for the beta variant.

In this analysis, the researchers evaluated volunteers enrolled in the phase 3 clinical trial who were immunized with two doses of CoronaVac in Chile. After administration of the second dose, serum samples were collected to measure the neutralizing capacity of the antibodies against the variants of concern. “It is important

to highlight that, after SARS-CoV-2 infection, the antibody blocking capacity of vaccinated volunteers increased for all variants tested,” scientists pointed out. According to them, immunization with CoronaVac in any regimen stimulates cellular responses against all variants of concern and contributes to neutralizing the infection caused by the virus.

Among 2,263 Chileans vaccinated with CoronaVac, only 45 developed Covid-19

The Immune Profile and Clinical Outcome of Breakthrough Cases After Vaccination with an inactivated SARS-CoV-2 Vaccine study, meanwhile, evaluated the safety, immunogenicity and efficacy of CoronaVac in preventing severe cases of Covid-19. From the 2,263 fully vaccinated individuals at the end of June 2021, only 45 (i.e., 1.99%) experienced symptoms of infection 14 days or more after the second dose.

Of these 45, 43 developed mild cases. The exceptions were two cases of men over 60. The first of them, a 62-year-old man with two comorbidities (hypothyroi-

dism and obesity), developed a mild case and required supplemental oxygenation. The second, a 69-year-old man with four comorbidities (obesity, hypertension, bicuspid aortic, and atrial fibrillation), developed a more severe condition and required mechanical ventilation. Both recovered and are well.

The researchers pointed out that vaccination with CoronaVac is effective. “The cases of the disease were mostly mild and did not necessarily correlate with the lack of vaccine-induced immunity, suggesting that other factors, to be defined in future studies, could lead to symptomatic infection after CoronaVac vaccination.”

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Recognition of Variants of Concern by Antibodies and T Cells Induced by a SARS-CoV-2 Inactivated Vaccine

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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

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 (B.1.351), 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 μ g (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 μ L 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 μ L 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 μ L of 3,3',5,5'-tetramethylbenzidine (TMB - BD #555214) was added. An equal volume of 2 N H₂SO₄ 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: $[\text{OD}_{450\text{nm}} \text{ value of negative control} - \text{OD}_{450\text{nm}} \text{ value of sample}] / [\text{OD}_{450\text{nm}} \text{ value of negative control} * 100]$. 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 $\geq 1/4$ 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% CO₂. 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 shown 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

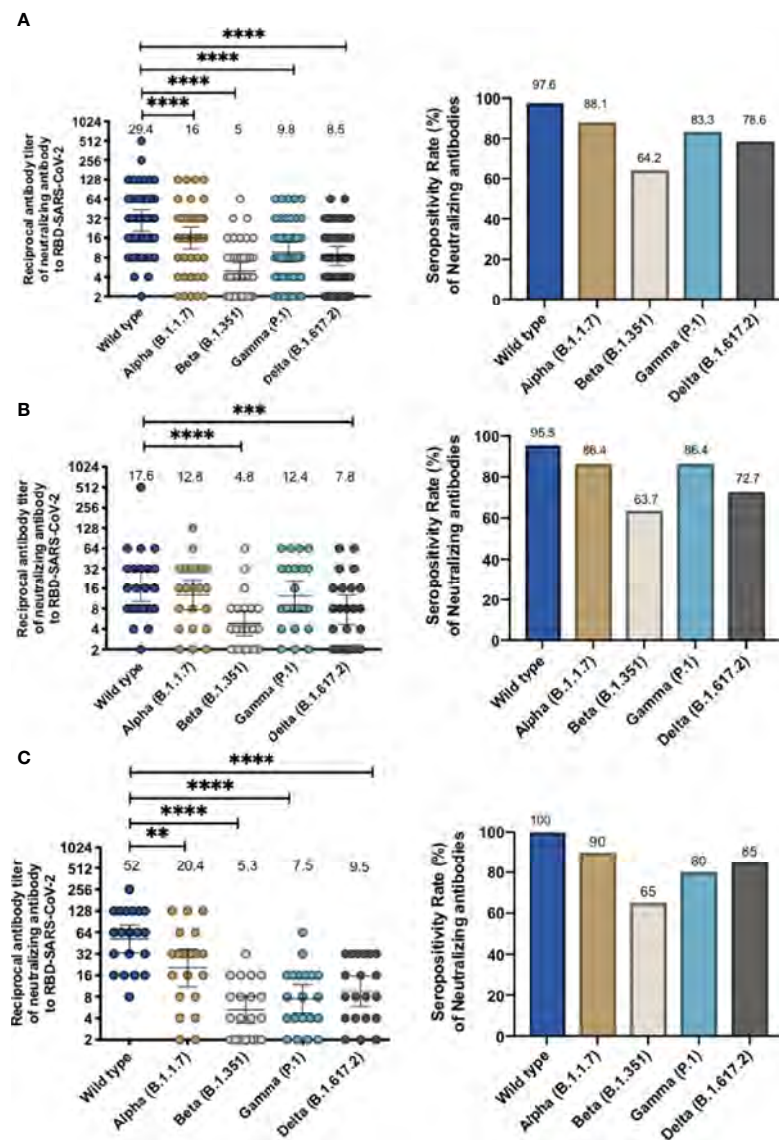


FIGURE 1 | Immunization with CoronaVac induces antibodies able to inhibit the interaction between hACE2 and S1-RBD from SARS-CoV-2 variants after two immunizations in a 0-14 and 0-28 schedule. Antibody titers were evaluated with a surrogate virus neutralization assay (sVNT), which quantifies the interaction 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

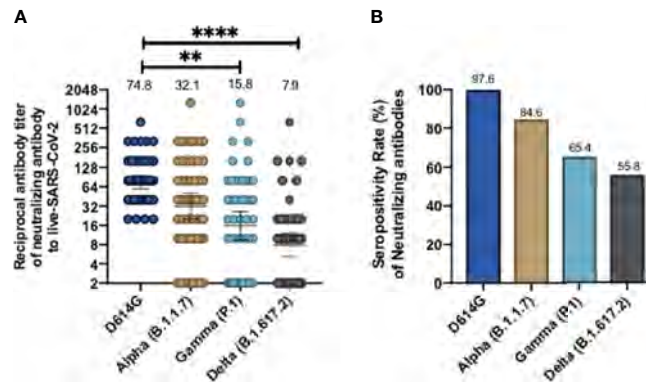


FIGURE 2 | CoronaVac immunization induces neutralizing antibodies against SARS-CoV-2 variants after two vaccine doses using a conventional virus neutralization test. Neutralizing antibody titers were evaluated by incubating the serum with a SARS-CoV-2 Chilean clinical strains and then added into Vero 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 (95% CI)	17.6 10.3-30.2	12.8 7.7-21.5	12.4 7.3-21.2	4.8 3.2-7.4	7.8 4.7-12.9	N/D (-)
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 (95% CI)	52.0 33.1-81.4	20.4 11.1-37.4	7.5 4.7-11.2	5.3 3.4-8.1	9.5 5.9-15.4	N/D (-)
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 (95% CI)	29.5 20.2-43.1	16.0 10.9-23.5	9.8 6.9-13.9	5.0 3.8-6.7	8.5 6.1-11.9	N/D (-)

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

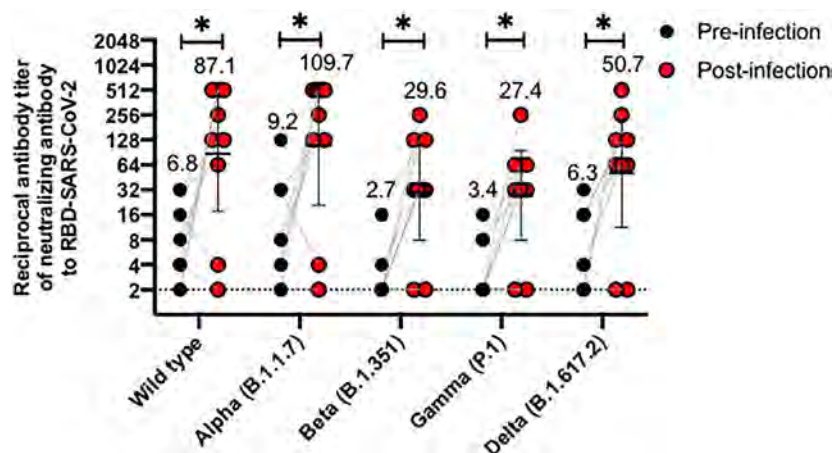


FIGURE 3 | CoronaVac immunization induces antibodies able to inhibit the interaction between hACE2 and S1-RBD from SARS-CoV-2 variants in vaccine breakthrough cases after two vaccine doses. Antibody titers were evaluated with a surrogate virus neutralization assay (sVNT), which quantifies the interaction 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.

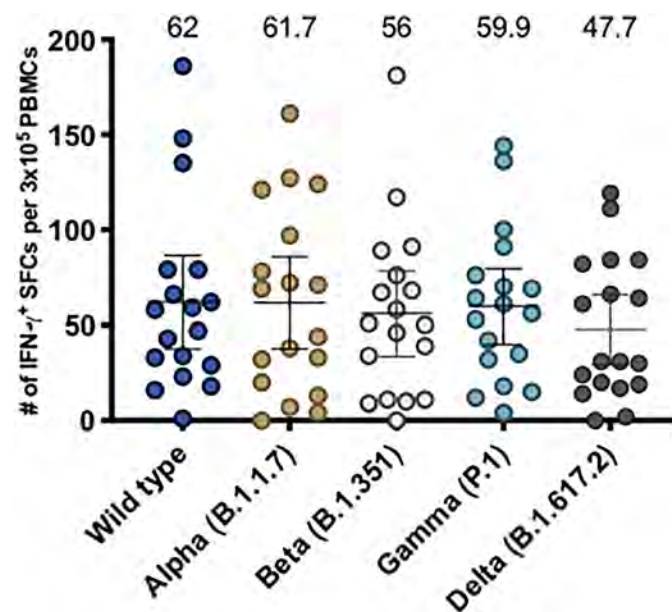


FIGURE 4 | Evaluation of cellular immune response through ELISPOT upon stimulation with Mega Pools of Spike peptides derived from SARS-CoV-2 WT and variants of concern in volunteers immunized with CoronaVac. Numbers of IFN- γ -secreting cells, determined through ELISPOT as spot forming cells (SFCs) were 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 \pm 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.

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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 viral cytopathic 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.

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La Jolla Institute for Immunology (LJI) has filed for patent protection for various aspects of T cell epitope and vaccine design work.

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Immune Profile and Clinical Outcome of Breakthrough Cases After Vaccination With an Inactivated SARS-CoV-2 Vaccine

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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 μ g (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 $\geq 94\%$ 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; PaO₂/FiO₂ < 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: $(\text{OD}_{450\text{ nm}} \text{ value of negative control} - \text{OD}_{450\text{ nm}} \text{ value of sample}) / (\text{OD}_{450\text{ nm}} \text{ value of negative control} \times 100)$, 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.

Two out of the nine breakthrough cases (Volunteers 4 and 7) reached a score over 2. The highest clinical score registered for Volunteer 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 pneumoniae* 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-

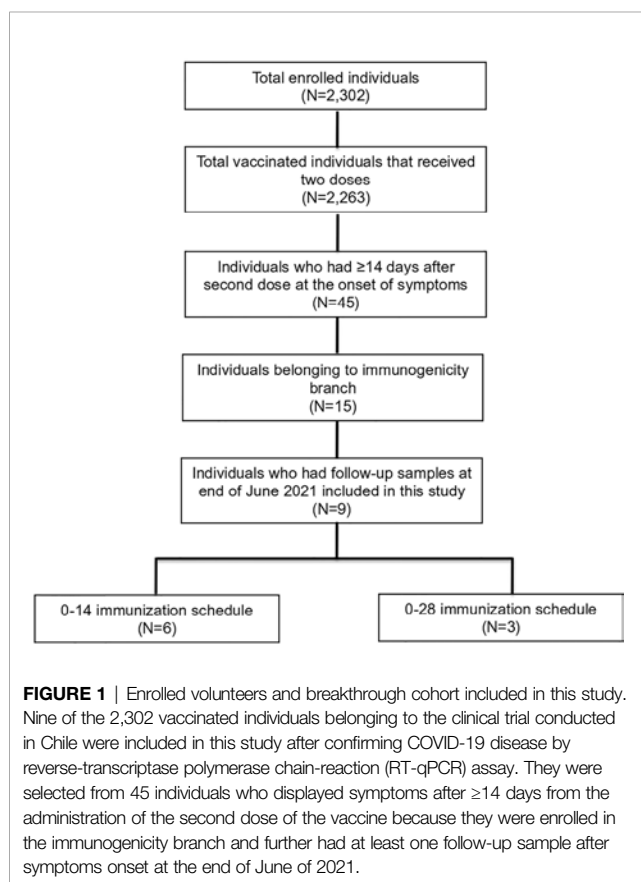


TABLE 1 | Demographic and clinical history of nine vaccine breakthrough cases.

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, nephrolithiasis
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

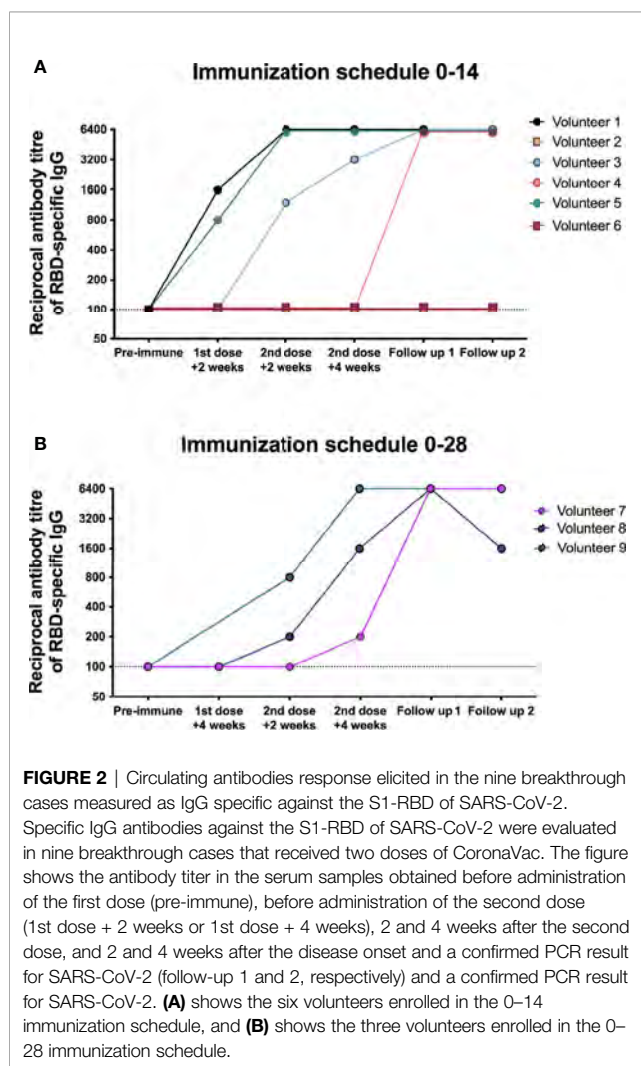


FIGURE 2 | Circulating antibodies response elicited in the nine breakthrough cases measured as IgG specific against the S1-RBD of SARS-CoV-2. Specific IgG antibodies against the S1-RBD of SARS-CoV-2 were evaluated in nine breakthrough cases that received two doses of CoronaVac. The figure shows the antibody 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- to 11-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**.

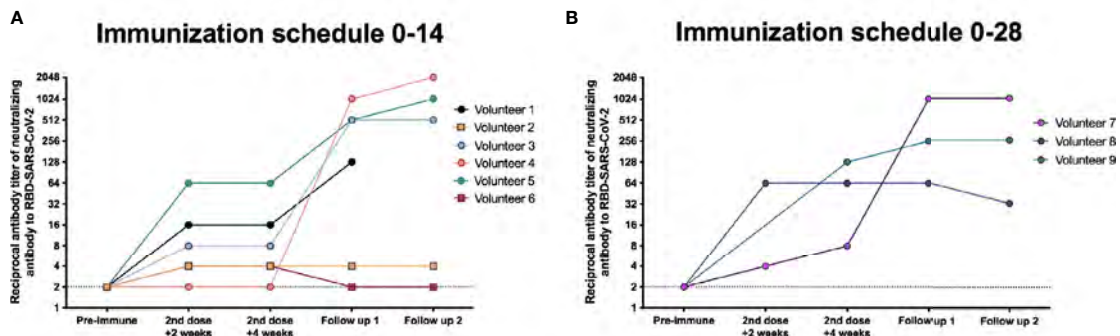
Overall, the results suggest that the cellular immune response elicited after either vaccination or infection in these nine breakthrough cases does not necessarily correlate with protection against SARS-CoV-2.

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**).

Surrogate Virus Neutralizing Testing (sVNT)



Conventional Virus Neutralizing Test (cVNT)

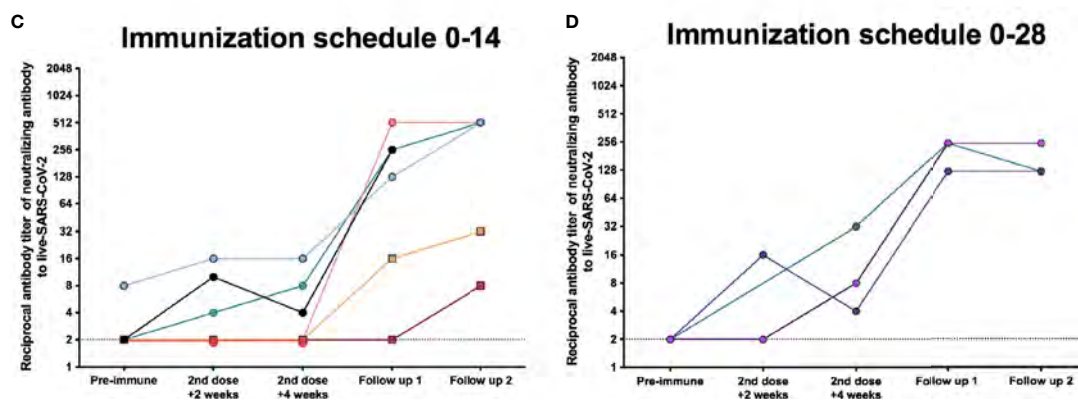


FIGURE 3 | Circulating antibodies exhibit varying neutralizing capacities in the nine breakthrough cases. Neutralizing antibodies were evaluated before administration of the first dose (pre-immune), 2 and 4 weeks after the second dose, and 2 and 4 weeks after the disease onset (follow-up 1 and 2, respectively). 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).

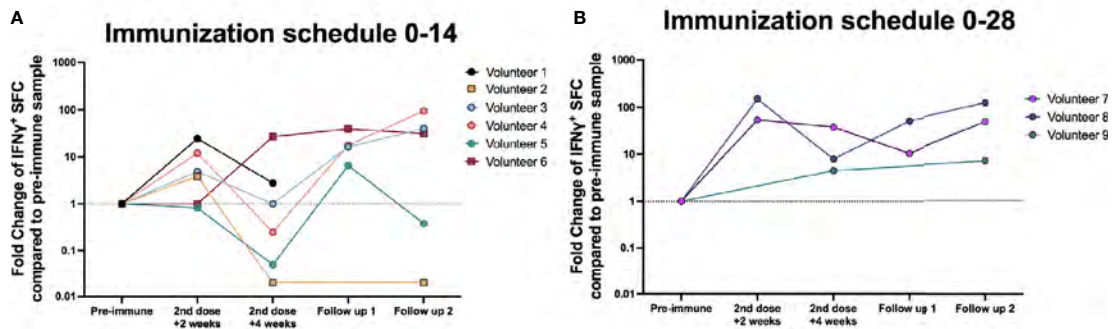
In summary, these results show that detection of low levels of neutralizing antibodies after vaccination could be related to symptomatic infection; however, unknown underlying conditions

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

Stimulation with 15-mer Megapools of SARS-CoV-2 Antigens



Stimulation with 9 to 11-mer Megapools of SARS-CoV-2 Antigens

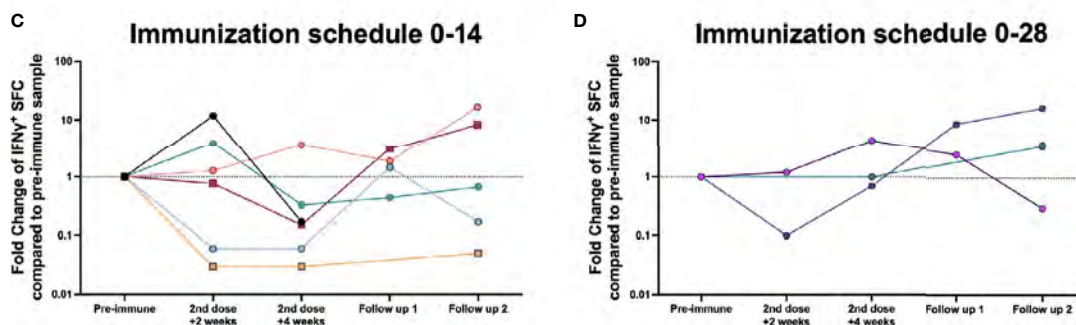


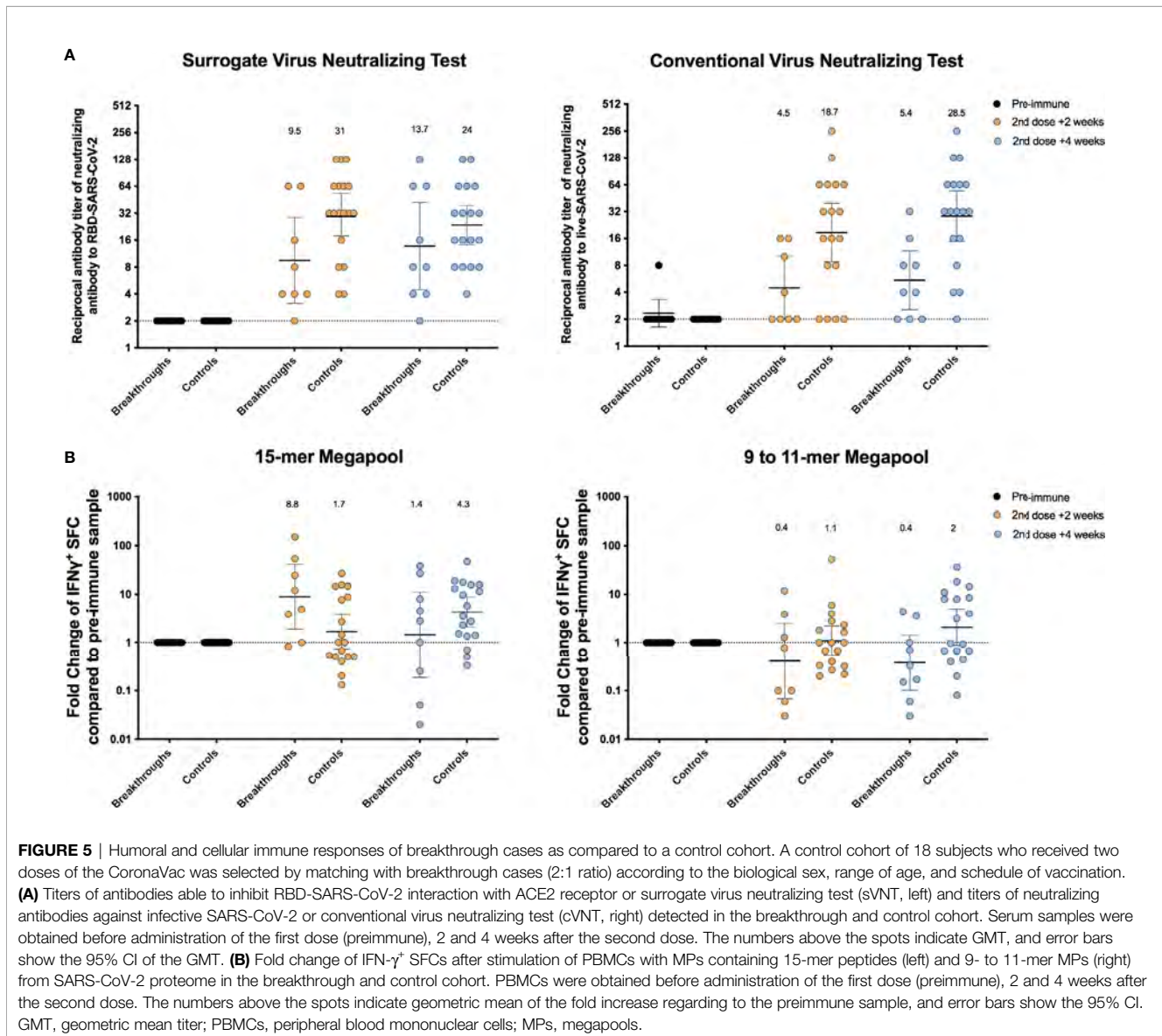
FIGURE 4 | The IFN- γ production by T cells from breakthrough cases after stimulation with MegaPools of SARS-CoV-2 peptides is heterogeneous. PBMCs from the nine breakthrough cases were obtained before administration of the first dose (pre-immune), 2 and 4 weeks after the second dose, and 2 and 4 weeks 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



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 adaptive 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 of 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 CoronaVac, 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.

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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.

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Conflict of Interest: ZG and MW are SINOVAAC 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.

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2.7. Study suggests that countries in South America that used CoronaVac are protected against gamma and lambda variants

A study from Brazil and Uruguay indicates that the population of southern countries from South America are more protected against the regional variants gamma and lambda of the virus SARS-CoV-2. The conclusions are in a scientific article published in MedRxiv. According to the researchers, from the Republic University of Montevideú, and from the Oswaldo Cruz Foundation (Fiocruz), from Rio de Janeiro, CoronaVac, a vaccine from Butantan and Sinovac, in the condition of inactivated vaccine, contributed decisively for that result.

According to the study, Argentina, Brazil, Chile, Paraguay and Uruguay experienced severe epidemic waves of Covid-19 in the beginning of 2021, boosted by the expansion of the variants gamma and lambda. However, beginning from June, there was an improvement in the indicators of the pandemic. In the 14th epidemiological week, between April 4th and 10th, there

were registered 21.141 deaths caused by Covid-19, according to the Coronavirus Panel of the Ministry of Health. It was the highest number of deaths in seven days during the whole year. However, on the 25th epidemiological week, between June 20th and 26th, the number of deaths had decreased to 11.935. Since then, the indicator kept decreasing and, in the last epidemiological week, between September 19th and 25th, the number of deaths caused by Covid-19 in Brazil was 3.692.

The study says that the generalized use of CoronaVac in the southern countries of South America was not just efficient to prevent the severe cases of Covid-19, but also contained the dissemination of the regional variants that were highly transmissible. In Chile, 70% of the vaccines applied corresponds to CoronaVac; in Uruguay, 60%; in Brazil, 35%. It's also worth pointing out that until the middle

of May, the vaccine from Butantan corresponded to about seven from 10 vaccines applied.

To investigate the results of national vaccination programs and the impact of the natural infection in the viral transmission of the southern countries, the researchers analyzed the association between population mobility and the effective number of reproduction (R_t) - average number of people infected by an infected person introduced in a partially immunized population or susceptible (It means, in the beginning of the pandemics).

The analysis revealed that, from January to May 2021, the mobility of the population in Argentina, Brazil, Chile, Paraguay and Uruguay were related to the effective number of reproduction R_t . However, from June, the index of viral transmission began to decrease more than expected according to the level of social interaction. “The study

suggests that the populations from the southern countries of South America probably reached the HIT (herd immunity threshold) to contain the transmission of variants gamma and lambda of SARS-CoV-2 around the middle of 2021”, affirmed the researchers. The results indicate that the immunity threshold HIT for the Covid-19 virus, in South America, varied among 29% in Argentina, 33% in Uruguay, 36% in Paraguay, 43% in Chile and 45% in Brazil.

The researchers suggest that the high level of natural immunity identified in the countries of South America may be an important condition that might be contributing to limit the transmission of the variant. According to the specialists, the contribution of that immunity is a result of the natural infection associated with the vaccination.

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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?

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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

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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 Amer-

ican 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

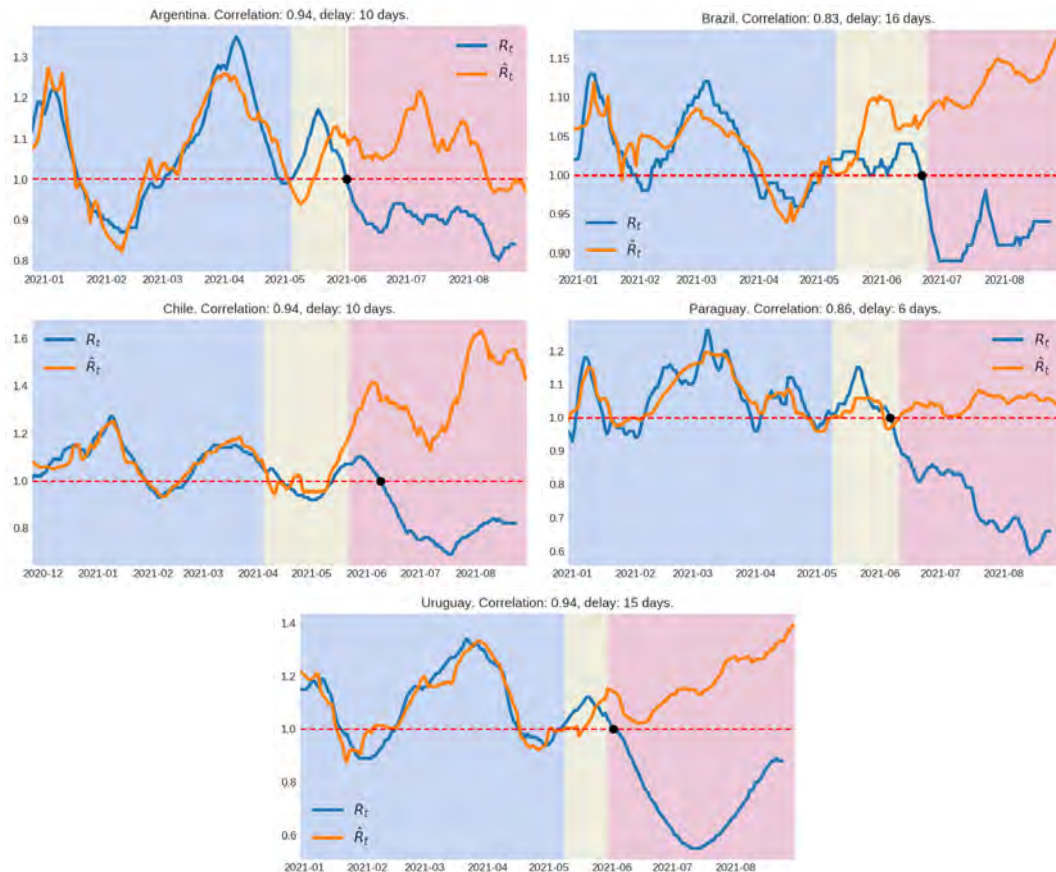


Figure 1: Viral effective reproduction number R_t and its estimation \hat{R}_t using mobility information. Background colors indicate the following time periods: in blue, the time period used to fit the linear model (see Section 4.2), in yellow, the period after the fitting, but before the decoupling 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-

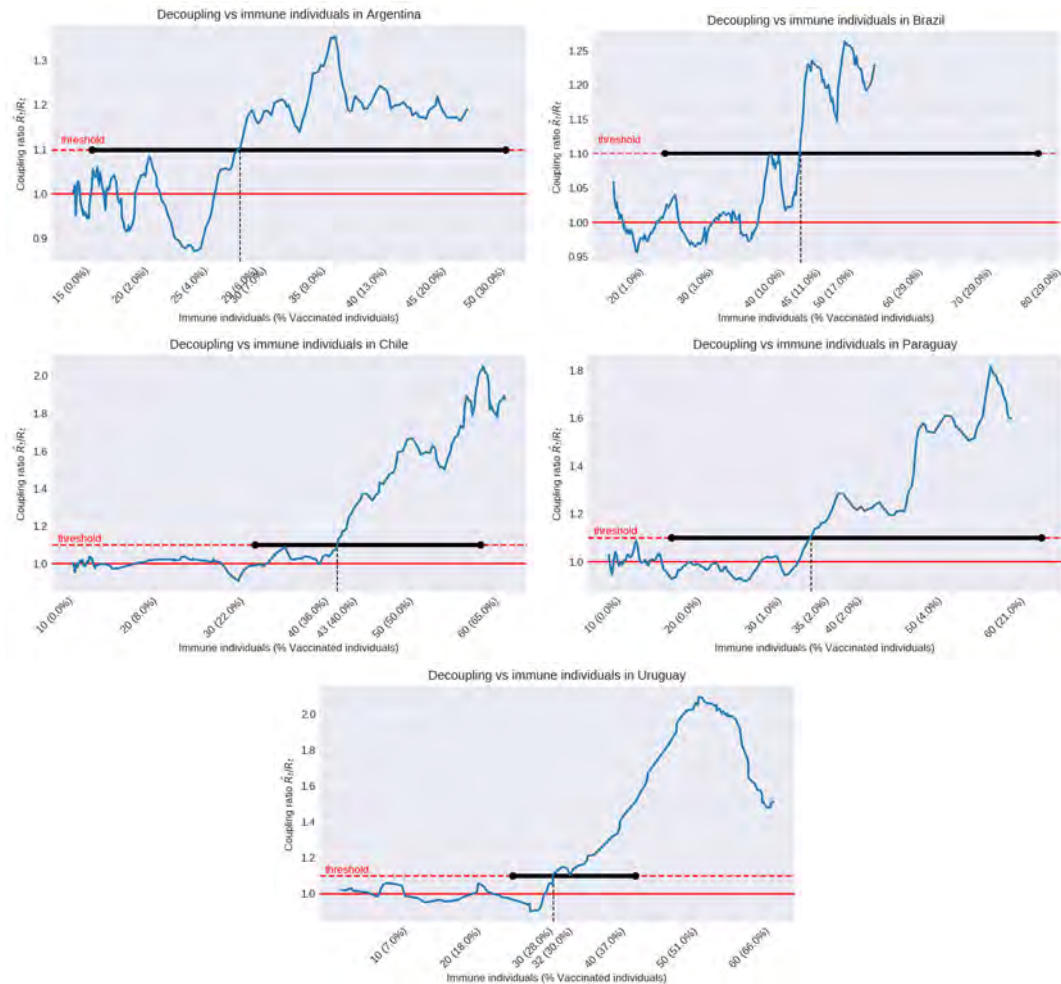


Figure 2: Coupling ratio \hat{R}_t/R_t plotted with respect to the percentage of immune population. During the first months of 2021 the coupling ratio varies around 1, which corresponds to the periods where the R_t and \hat{R}_t are in concordance in Figure 1. Immune population includes immunity achieved 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 than 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 administered 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). Vaccination 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 (≥ 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 vaccination 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.

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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 assumption 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 be greater than the percentage of immunized population.

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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.

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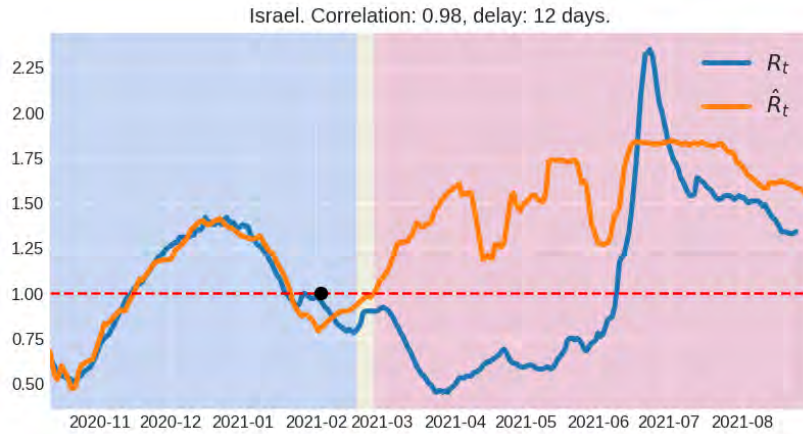


Figure A.1: Viral effective reproduction number R_t and its estimation \hat{R}_t using mobility information. Background colors indicate the following time periods: in blue, the time period used to fit the linear model (see Section 4.2), in yellow, the period after the fitting, but before the decoupling 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.

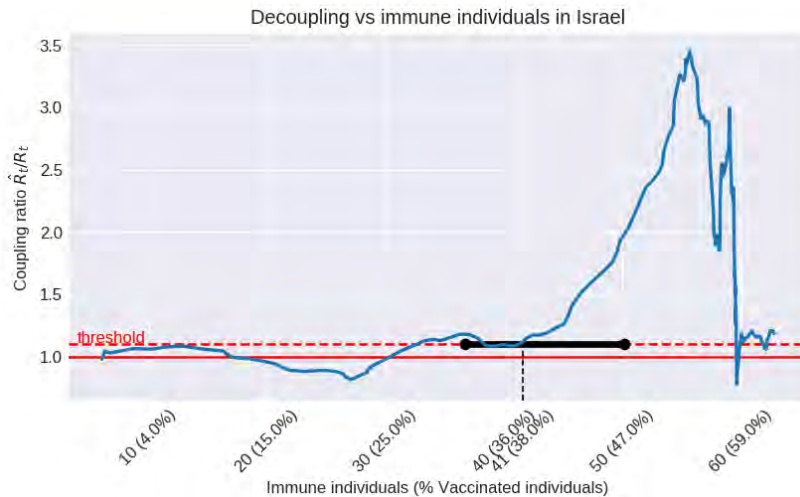


Figure A.2: Coupling ratio \hat{R}_t/R_t plotted with respect to the percentage of immune population. During the first months of 2021 the coupling ratio varies around 1, which corresponds to the periods where the R_t and \hat{R}_t are in concordance in Figure A.1. Immune population includes immunity 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

What science proves

2.8. Booster dose of CoronaVac increases in 17 times the level of antibodies capable of combating the delta variant of SARS-CoV-2, says study

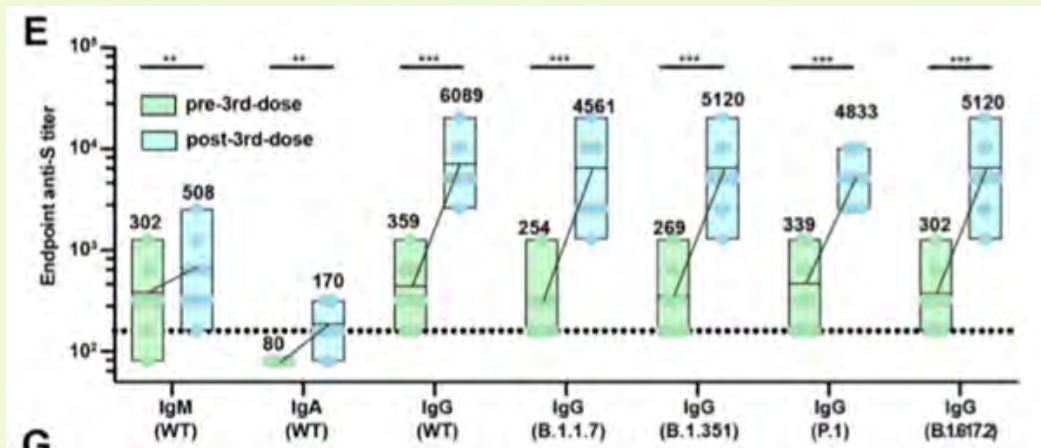
A booster dose of CoronaVac, a vaccine from Butantan and the Chinese pharmaceutical Sinovac against Covid-19, increases in 17 times the level of neutralizing antibodies against the delta variant of the SARS-CoV-2 virus on those that have completed the vaccinal scheme for six months. The conclusions are in the study “A third dose of inactivated vaccine augments the potency, breadth, and duration of anamnestic responses against SARS-CoV-2”, from researchers of the Chinese Science Academy, University of Beijing, Medical School of Shanghai and Sinovac, among other institutions, published in MerRxiv platform.

The study demonstrated that the booster dose of CoronaVac quickly potencialize the level of neutralizing antibodies against the Spike protein, a component that the virus uses to invade the human cells. Besides that, the booster dose also increases in 17 times the level of neutralizing antibodies against the

original virus (strain from Wuhan); in 18 times against the alfa variant; in 19 times against the beta variant; and in 14 times against gamma.

The research analyzed plasma samples from 66 participants, including 38 volunteers that received two or three doses of the vaccine. The evaluation happened four weeks after the administration of the booster dose, being this one applied six months after the participants received the second dose.

The graphic shows the increasing level of antibodies in the participants, measured immediately before they receive the booster dose of CoronaVac (in green), and four weeks after the booster dose (in blue). The results shown are from the original virus from Wuhan (WT, initials for “wild type”), and from each one of the four variants of concern: alfa (B.1.1.7), beta (B.1.351), gamma (P.1) and delta (B.1.617.2).



CoronaVac has already shown to be efficient against the gamma variant in the study of effectiveness of Project S, conducted by Butantan in Serrana, São Paulo. In this study, 95% of the adult population was vaccinated with CoronaVac between 2021 February and 2021 April, when the variant gamma was already predominant in Brazil. The collective immunization made the deaths caused by Covid-19 decrease in 95%, the hospitalizations in 86% and the symptomatic cases in 80%.

Another research from China demonstrated the efficacy of CoronaVac against the delta variant. A study of the Center of Disease Control and Prevention from the Guangdong province, made during an outbreak of Covid-19 caused by delta variant, showed that CoronaVac avoided the development of severe cases of Covid-19 and had the efficacy of 69,5% against the emergence of pneumonias due to the disease. The study was conducted with 10.813 people between 2021 May and 2021 June.

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1 **A third dose of inactivated vaccine augments the potency, breadth,**
2 **and duration of anamnestic responses against SARS-CoV-2**

3
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32 **NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.**

33 **Abstract: (~150 words)**

34 Emergence of variants of concern (VOC) with altered antigenic structures and waning
35 humoral immunity to SARS-CoV-2 are harbingers of a long pandemic. Administration
36 of a third dose of an inactivated virus vaccine can boost the immune response. Here,
37 we have dissected the immunogenic profiles of antibodies from 3-dose vaccinees, 2-
38 dose vaccinees and convalescents. Better neutralization breadth to VOCs, expeditious
39 recall and long-lasting humoral response bolster 3-dose vaccinees in warding off
40 COVID-19. Analysis of 171 complex structures of SARS-CoV-2 neutralizing
41 antibodies identified structure-activity correlates, revealing ultrapotent, VOCs-
42 resistant and broad-spectrum antigenic patches. Construction of immunogenic and
43 mutational heat maps revealed a direct relationship between “hot” immunogenic sites
44 and areas with high mutation frequencies. Ongoing antibody somatic mutation,
45 memory B cell clonal turnover and antibody composition changes in B cell repertoire
46 driven by prolonged and repeated antigen stimulation confer development of
47 monoclonal antibodies with enhanced neutralizing potency and breadth. Our findings
48 rationalize the use of 3-dose immunization regimens for inactivated vaccines.

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52 **One sentence summary**

53 A third booster dose of inactivated vaccine produces a highly sifted humoral immune
54 response *via* a sustained evolution of antibodies capable of effectively neutralizing
55 SARS-CoV-2 variants of concern.

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60 **Main Text:**

61 The ongoing coronavirus disease 2019 (COVID-19) pandemic caused by severe
62 acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has lasted for one and a
63 half years, resulting in an unprecedented public health crisis with over 4 million
64 deaths globally. Progress in halting this pandemic seems slow due to the emergence
65 of variants of concern (VOC), such as the B.1.1.7 ([Alpha](#)), B.1.351 ([Beta](#)), P.1
66 ([Gamma](#), also known as B.1.1.28.1) and more recent B.1.617.2 ([Delta](#)), that appear
67 to be high transmissible and more resistant to neutralizing antibodies ([1-4](#)). While
68 several types of COVID-19 vaccines are being deployed at a large scale, new variants
69 are thought to be responsible for re-infections, either after natural infection or after
70 vaccination, as observed in Brazil and the United States, respectively ([5, 6](#)). Closely
71 correlated with these, a general decrease in immune protection against SARS-CoV-
72 2 variants within 6-12 months after the primary infection or vaccination is also
73 observed ([6-8](#)). The prospect of genetic recombination and antigenic drift in recent
74 SARS-CoV-2 variants together with non-uniform immune protections arising from
75 heterogeneously waning humoral immunity in COVID-19 convalescent or
76 vaccinated individuals, point to the potential risks of a long-term pandemic that could
77 endanger the global human health, diminishing social, economic and outdoor leisure
78 activities. A plausible approach to solving this problem is the administration of a
79 third dose of the vaccine somewhere between 6 and 12 months after the 2nd dose of
80 vaccination for enhancing and prolonging the protection. However, not much is
81 known about the immunogenic features of such a booster dose of a COVID-19 vaccine.
82 In addition, there are large gaps in our understanding about correlating immunogenic
83 findings from surrogate endpoints to gauge vaccine efficacy.

84

85 The CoronaVac, a 2-dose β -propiolactone-inactivated vaccine against COVID-19,
86 has been approved for emergency use by the World Health Organization ([9, 10](#)). In
87 human clinical trials (phase I/II, registration number: NCT04352608), a subgroup
88 with a 3-dose immunization schedule at months 0, 1, 7 was also included. To evaluate
89 immune features, we recruited 22 COVID-19 convalescents, 6 healthy participants
90 (SARS-CoV-2 negative, confirmed by RT-PCR) and 38 volunteers who received

91 either 2 or 3 doses of the Coronavac vaccine for blood donation. The volunteers
92 ranged from 16 to 69 years old (median 33); 30 (45.5%) were men and 36 (54.5%)
93 were women. None of the volunteers recruited for vaccination was infected by
94 SARS-CoV-2 prior to the study. Blood samples from convalescents and vaccinees
95 collected 1.3 months after infection and the indicated times after vaccination were
96 used in this study, respectively, to compare humoral immune responses elicited
97 against circulating SARS-CoV-2 variants.

98

99 Neutralizing antibodies (NAbs) are a major correlate of protection for many viruses,
100 including SARS-CoV-2, and have also provided the best correlate of vaccine
101 efficacy. Several types of SARS-CoV-2 neutralization assays have been described
102 using either live SARS-CoV-2 or a pseudo-typed reporter virus carrying SARS-CoV-
103 2 spike protein (S). Both types of assays could yield reproducible neutralizing titers,
104 with the pseudo-typed virus neutralization assay exhibiting higher sensitivity (11,
105 12). Neutralizing activity of plasma samples from 66 participants was measured
106 against WT, B.1.351, P.1 and B.1.617.2 using live SARS-CoV-2 and VSV-
107 pseudoviruses with the S from WT, B.1.1.7, P.1 variants and SARS-CoV (Fig. 1).
108 The geometric mean half-maximal neutralizing titers (GMT NT₅₀) against live
109 SARS-CoV-2 in plasma obtained from convalescents and from vaccinees (4 weeks
110 after the final vaccination) suggest an approximately 60% higher neutralizing
111 activity against WT after 3-dose inoculation when compared with 2-dose
112 administration, and 20% higher than those from convalescents (Fig. 1A).
113 Interestingly, for the samples from the convalescents, 2-dose and 3-dose vaccinees,
114 neutralizing titers against B.1.351 were, on average, 7.7-fold, 5.7-fold and 3.0-fold
115 reduced, respectively, compared with WT (Fig. 1A). Similarly, fold decreases in
116 neutralization ID₅₀ titers against P.1 and B.1.617.2 for the three cohorts were 5.3, 4.3
117 and 3.1, and 5.3, 3.7 and 2.3, respectively (Fig. 1A). Overall, plasma of the 3-dose
118 vaccinees displayed minimal reduction in neutralization titers against several
119 authentic VOCs compared to the convalescents and 2-dose vaccinees (Fig. 1A).
120 Remarkably, ~41% (9/22) and 50% (6/12) samples from the convalescents and 2-
121 dose vaccinees, respectively, failed to reach 50% neutralization at a plasma dilution

122 of 1: 10, with ~14% (3/22) and 16% (2/12) showing a near ineffectiveness in
 123 neutralizing B.1.351 *in vitro* (Fig. 1A). By contrast, only 1 out of 14 samples from
 124 the 3-dose vaccinees exhibited a weak neutralizing titer below 10 (Fig. 1A).
 125 Importantly, the 3-dose vaccinees showed over 2.5-fold higher neutralizing potency
 126 against B.1.617.2 than the convalescents and 2-dose vaccinees (Fig. 1A). The GMT
 127 NT₅₀ values measured by a VSV-pseudovirus with the WT S were 840, 660 and 1,176
 128 for convalescents, 2-dose and 3-dose vaccinees, respectively, which were 8-10-fold
 129 greater than those determined by live WT SARS-CoV-2 (Fig. 1A, 1B), confirming
 130 higher sensitivity of pseudovirus-based assays in determining neutralizing titers. In
 131 line with the results of live SARS-CoV-2 neutralization assay, the mean fold decrease
 132 in the neutralization of B.1.1.7 relative to the WT was 2.8-fold for convalescents,
 133 2.2-fold for 2-dose vaccinees and 1.7-fold for 3-dose vaccinees (Fig. 1B). Similarly,
 134 plasma from convalescents, 2-dose and 3-dose vaccinees exhibited a 4.5-fold, 2.9-
 135 fold and 2.4-fold reduction, in NAb titers against P.1, respectively, when compared
 136 to the WT (Fig. 1B). These results reveal that a third-dose boost of inactivated
 137 vaccine leads to enhanced neutralizing breadth to SARS-CoV-2 variants, bolstering
 138 the potential to ward off VOCs effectively when compared to convalescent plasma.
 139 Of note, neither vaccination nor SARS-CoV-2 infection boosts distinct neutralizing
 140 potency against SARS-CoV, presumably due to the relatively far phylogenic
 141 relationship (Fig. 1B).

142

143 To seek information on potential binding-neutralization correlates, the abilities of
 144 antibodies present in plasma to bind the receptor-binding domain (RBD), N-terminal
 145 domain (NTD), S-trimer and nucleoprotein (N) from SARS-CoV-2 and its variants
 146 were measured by enzyme-linked immunosorbent assay (ELISA). As expected, all
 147 COVID-19 convalescents and vaccinees exhibited high anti-RBD, anti-NTD, anti-S
 148 and anti-N titers for SARS-CoV-2 variants, but weak antibody reactivity to SARS-
 149 CoV (Fig. 1C and fig. S1). Unexpectedly, the amount of N-specific IgG elicited by
 150 2-dose and 3-dose vaccination schedules was 2-6-fold lower than those of
 151 convalescents, and 2-6-fold lower than the antibodies targeting S or RBD in
 152 vaccinees, reflecting distinct serological profiles (Fig. 1C and fig. S1). Overall

153 plasma neutralizing activity against the WT was substantially correlated with anti-S
154 and anti-RBD binding titers in ELISA. However, only marginal correlates between
155 binding and neutralization potency were established for VOCs (fig. S2). In spite of
156 this, a 3-dose administration elicits a broader range of antibody binding activities to
157 VOCs with minimal decreasing folds than those of 2-dose vaccination and
158 convalescents (Fig. 1D and fig. S2).

159

160 To evaluate the nature of humoral immune response elicited by a booster dose of
161 CoronaVac, the S-specific IgA, IgM and IgG titers and neutralizing activities against
162 SARS-CoV-2 variants were monitored before and 4 weeks after the third
163 immunization. S-specific IgM and IgA titers were generally lower and were not
164 significantly boosted in response to the third-dose vaccination (Fig. 1E). Similar to
165 most convalescents (2), approximately 80~90% of both anti-S IgG and NAb titers
166 against the WT waned 6 months after the second vaccination (13), while the third-
167 dose administration of CoronaVac boosted these titers by ~20-fold at 4 weeks post
168 vaccination (Fig. 1E and F). Significantly, vaccinees 6 months after the second
169 immunization did not have detectable *in vitro* neutralizing activities against B.1.351,
170 P.1 and B.1.617.2, while all vaccinees exhibited a robust recall humoral response to
171 efficiently neutralize circulating variants post the third-dose vaccination (Fig. 1E and
172 F). To further characterize the expeditiousness, longevity and immunological
173 kinetics of recall response stimulated by the third-dose immunization, neutralizing
174 potencies at days 0, 7, 14, 28, 90 and 180 post the third-dose vaccination were
175 determined (Fig. 1G and H). Remarkably, NAb titer surged by ~8-fold (from 7 to 53)
176 at week 1, peaked by ~25-fold increase (up to 177) at week 2 after the 3rd-booster
177 and slowly decreased over time (Fig. 1G). Notably, NAb titer was maintained at
178 around 60 on 180 days post the 3rd-booster, comparable to the high level of NAb titer
179 elicited by the 2-dose administration (Fig. 1H). Taken together, these serological
180 results reveal a third-dose booster can elicit an expeditious, robust and long-lasting
181 recall humoral response.

182

183 The molecular mechanism underlying these potent, broad and durative antibody

184 responses elicited by a third-dose booster 6 months after the administration of the
 185 second dose of the vaccine, might involve ongoing antibody somatic mutation and
 186 evolution of antibody by affinity maturation through prolonged and repeated antigen
 187 stimulation (14, 15). Although circulating antibodies derived from plasma cells wane
 188 over time, long-lived immune memory can persist in expanded clones of memory B
 189 cells (16). Thereby, we used flow cytometry to sort the SARS-CoV-2 S-trimer-
 190 specific memory B cells from the blood of seven selected CoronaVac vaccinees,
 191 including four samples from 3-dose vaccinees and three samples from 2-dose
 192 vaccinees (Fig. 2A and fig. S3). The averaged percentage of S-binding memory B
 193 cells in 3-dose vaccinees was substantially greater than those in 2-dose vaccinees
 194 (Fig. 2A and fig. S3). Due to differences in labeling strategies employed for sorting
 195 SARS-CoV-2-specific B cells, the above percentage of memory B cells was not
 196 directly comparable with those reported in naturally infected individuals and in
 197 mRNA vaccinated individuals. The gated double-positive cells were single cell
 198 sorted and immunoglobulin heavy (*IGH*; IgG isotype) and light (*IGL* or *IGK*) chain
 199 genes were amplified by nested PCR. Overall, we obtained 422 and 132 paired heavy
 200 and light chain variable regions from S-binding IgG⁺ memory B cells from four 3-
 201 dose and three 2-dose vaccinees, respectively (Fig. 2B and fig. S4). Surprisingly,
 202 expanded clones of cells comprised 45-61% of the overall S-binding memory B
 203 compartment in 3-dose vaccinees, which is approximately 2-fold higher than those
 204 in COVID-19 convalescents and in mRNA or 2-dose vaccinated individuals (Fig. 2B
 205 and C). When compared to 2-dose vaccinees, the increase in the number of persistent
 206 clones and various clonal compositions in 3-dose vaccinated group suggested an
 207 ongoing clonal evolution (Fig. 2B and C). Shared antibodies with the same
 208 combination of *IGHV* and *IGLV* genes in 3-dose vaccinees comprised ~20% of all
 209 the clonal sequences. Similar to natural infection and mRNA vaccination (2, 14, 16),
 210 *IGHV3-30*, *IGHV3-53* and *IGHV1-69* remained significantly over-represented in 3-
 211 dose vaccinees (fig. S5). Meanwhile, notable differences in the frequency of human
 212 V genes between 3-dose vaccinated and the other two groups were observed as well
 213 (fig. S5). In 3-dose vaccinees, *IGHV3-21*, *IGHV4-39* and *IGHV7-4-1* were largely
 214 abundant, but *IGHV5-51*, *IGHV3-66* and *IGHV1-2* were significantly scarce when

215 compared to the other two groups (fig. S5), indicative of memory B cell clonal
216 turnover. Notably, large-scale, single-cell sequencing datasets generated from two
217 cohorts of 2-dose, 3-dose vaccinees and a group of convalescents revealed no distinct
218 preference in the frequency of *V* genes at total B cell repertoire level (fig. S6),
219 suggesting that a large abundance of antibodies with low expression or affinities exist
220 in B cells. Additionally, the number of nucleotide mutations in the *V* gene in 3-dose
221 vaccinees is higher than those in both 2-dose vaccinees and naturally infected
222 individuals assayed after 1.3 and 6.2 months, but slightly lower than those in
223 convalescent individuals 1 year after infection (Fig. 2D), revealing ongoing somatic
224 hypermutation of antibody genes. There was no significant difference in the length
225 of the IgG CDR3 between vaccinated (either mRNA or inactivated) and convalescent
226 (after 1.3 or 6.2 or months) groups (fig. S7). These results reveal that a third-dose
227 booster 6 months after the second vaccination elicits an enhanced and anamnestic
228 immune response, which is led by clonal evolution of memory B cell and ongoing
229 antibody somatic mutations, resulting in enhanced neutralizing potency, breadth and
230 longevity of the immune response against SARS-CoV-2.

231

232 To further explore the immunogenic characteristics of the antibodies obtained from
233 memory B cells in 3-dose vaccinees, 48 clonal antibodies, designated as XGv01 to
234 XGv50 (no expression for XGv37 and XGv48) were expressed and their antigen
235 binding abilities verified by ELISA (fig. S8). Biolayer interferometry affinities (BLI)
236 measurements demonstrated that all antibodies bound to WT SARS-CoV-2 at sub-
237 nM levels (fig. S9 and table S1). The normalized geometric mean ELISA half-
238 maximal concentration (EC_{50}) revealed that all antibodies ($EC_{50}=4.5$ ng/ml) obtained
239 from 3-dose vaccinees, in particular RBD-specific mAbs ($EC_{50}=3.5$ ng/ml),
240 possessed higher binding activities than RBD-mAbs from early convalescents (at 1.3
241 and 6.2 months after infection, $EC_{50}=5.0$ and 6.8 ng/ml, respectively) and mRNA
242 ($EC_{50}=4.4$ ng/ml) vaccinated individuals (2, 14-18), but were comparable to those
243 from late convalescent individuals ($EC_{50}=2.6$ ng/ml) assessed at 12 months after
244 infection (Fig. 2E). These results indicate the possibility of the loss of antibodies
245 with low binding affinities over time or an ongoing increase in affinity under the

246 repeated exposures of antigen. Among these antibodies tested, 26 bound to RBD, 16
 247 targeted NTD, and 6 interacted with neither RBD nor NTD, but bound S1 (S1/non-
 248 RBD-NTD) (fig. S9 and table S1). Pseudovirus neutralization assay revealed that all
 249 RBD-specific antibodies, 10 (~60%) of the 16 NTD-directed antibodies and 3
 250 (~50%) of the 6 S1/non-RBD-NTD antibodies were neutralizing, presenting a
 251 relatively high ratio for NAbs (Fig. 2F, fig. S10 and table S2). Authentic SARS-CoV-
 252 2 neutralization assay results largely verified their neutralizing activities, albeit with
 253 that higher concentrations were required for some NAbs (fig. S11). Compared to
 254 RBD antibodies, many NTD NAbs exhibited very limited neutralizing activities.
 255 Notably, approximately 30% of RBD antibodies showed extra potent activities with
 256 half-maximal inhibitory concentration values (IC_{50}) < 0.1 nM. In line with binding
 257 affinity, the normalized geometric mean IC_{50} of the RBD antibodies of 3-dose
 258 vaccinees was 80 ng/ml, substantially lower than those from naturally infected
 259 individuals (ranging from 1.3 to 6.2 months, IC_{50} =130-160 ng/ml) and mRNA
 260 vaccinated individuals (IC_{50} =150 ng/ml), but similar to those from late convalescents
 261 (IC_{50} =78 ng/ml) (Fig. 2E) (2, 14-18). The overall increased neutralizing potency
 262 might have resulted from the ongoing accumulation of clones expressing antibodies
 263 with tight binding and potent neutralizing activities. Our experimental observations
 264 are consistent with a more recent study where antibodies generated from clonal B
 265 cells after 12 months showed enhanced neutralizing activities (14, 15).

266
 267 To examine the cross-reactivity against VOCs and other human coronaviruses,
 268 binding responses of these antibodies to WT, B.1.1.7, P.1, B.1.351, B.1.617.2, SARS-
 269 CoV, HuCoV NL63, HuCoV 229E and HuCoV HKU1 were measured. All but 2 of
 270 the 48 antibodies showed strong cross-binding to SARS-CoV-2 VOCs and about one-
 271 third of antibodies exhibited clear cross-reactivity to SARS-CoV, but none of these
 272 bound to HuCoV NL63, HuCoV 229E or HuCoV HKU1 (fig. S12). For ~ 20% and
 273 25% of RBD- and NTD-targeting antibodies, respectively, binding affinities against
 274 B.1.351/B.1.617.2 were over 10-fold reduced compared with WT (Fig. 2E). To
 275 further determine the neutralization breadth, the neutralizing activity of these
 276 antibodies was assayed against five VOCs and SARS-CoV. Out of 26 RBD NAbs,

277 24 possessed cross-neutralization activity against all five SARS-CoV-2 VOCs (Fig.
278 2F and fig. S13). Among these, six RBD antibodies could cross-neutralize SARS-
279 CoV, of which 2 exhibited more potent neutralization activity against SARS-CoV
280 with IC₅₀ values of 41 and 73 ng/ml. However, most of the NTD and S1/non-RBD-
281 NTD NAbs lost their abilities to inhibit viral infection (Fig. 2F and fig. S13),
282 indicative of higher variations for the NTD in VOCs. In comparison with NAbs from
283 early convalescents, antibodies isolated from 3-dose vaccinees showed overall
284 enhanced neutralizing potency and breadth to VOCs.

285

286 RBD is one of the main targets of neutralization in SARS-CoV-2 and other
287 coronaviruses. Due to its inherent conformational flexibility, RBD exists in either an
288 “open” (ACE2 receptor accessible) or “closed” (ACE2 receptor inaccessible)
289 configuration (19, 20), bearing antigenic sites with distinct “neutralizing sensitivity”.
290 To dissect the nature of the epitopes of RBD targeted by NAbs, 171 SARS-CoV-2
291 RBD-targeting NAbs with available structures (2, 15, 21-82), including 8 cryo-EM
292 structures determined in this manuscript (fig. S14-S15 and table S3), were examined.
293 By using cluster analysis on epitope structures, the antibodies were primarily
294 classified into six sites (I, II, III, IV, V and VI) (Fig. 3A and fig. S16), that are related
295 to the four or five classes assigned in recent studies (22, 31). Additionally, we
296 superimposed structures of RBDs from these complex structures and calculated the
297 clash areas between any 2 NAbs (Fig. 3B). Both strategies yielded identical results.
298 Combining the results of the characterization of binding and neutralization studies
299 reported previously with those determined here, the key structure-activity correlates
300 for the six classes of antibodies were analyzed (Fig. 3). Antibodies with sites I, II and
301 III, most frequently elicited by SARS-CoV-2 early infection, target the receptor-
302 binding motif (RBM), and potently neutralize the virus by blocking the interactions
303 between SARS-CoV-2 and ACE2 (Fig. 3C and D). Class I antibodies, mostly derived
304 from *IGHV3-53/IGHV3-66* with short HCDR3s (generally <15 residues), recognize
305 only the “open” RBD, and make contact with K417 and N501, but not
306 L452/T478/E484 (Fig. 3C and D, and fig. S16-S17). Notably, mutations such as
307 K417N, L452R, T478K, E484K and N501Y, or combinations of these mutations,

308 identified in several VOCs like B.1.1.7, B.1.617.2, P.1 and B.1.351, have been
 309 demonstrated to be key determinants for the viral escape of neutralization by many
 310 NAbS (fig. S18) (1, 81). Approximately ~75% and 60% of class I NAbS were
 311 significantly impaired in binding and neutralizing activities against B.1.351 as well
 312 as P.1, respectively, due to the combined mutations of K417N/T and N501Y (Fig. 3D
 313 and E, and fig. S18). Contrarily, Class III antibodies that are encoded by *IGHV1-2*
 314 and other variable heavy (VH)-genes and bound to RBD either in “open” or “closed”
 315 conformation, extensively associate with E484, and partially with L452, but not
 316 K417/T478/N501 (Fig. 3D and fig. S17C). Interestingly, *IGHV3-53/IGHV3-66* RBD
 317 antibodies with long HCDR3s (>15 residues) switch their epitopes from the site I to
 318 site III, indicating a clear antigenic drift during the process of somatic
 319 hypermutations (fig. S17C). Disastrously, over 90% class III antibodies showed a
 320 complete loss of activity against B.1.351 as well as P.1 largely owing to an E484K
 321 mutation (Fig. 3E). Against B.1.617.2, the substantially decreased activity of ~half
 322 of the class III antibodies is presumably mediated by L452R (Fig. 3E). Class II
 323 antibodies use more diverse VH-genes and target the patch lying between sites I and
 324 III (Fig. 3D and fig. S19). Surprisingly, antibodies binding to site II possess relatively
 325 lower specificity in recognition of epitope clusters ranging from K417, L452, S477,
 326 E484 to N501 (fig. S16). Like site I, site II can only be accessed when the RBD is in
 327 “open” conformation (Fig. 3A). As expected, the effects of mutations on the activity
 328 of class II antibodies were severe, two-thirds of these antibodies had >10-fold fall in
 329 neutralization activities against VOCs (Fig. 3E). Overall, the above analysis reveals
 330 that the RBD mutations identified in several VOCs can significantly reduce and, in
 331 some cases, even abolish the binding and neutralization of classes I to III antibodies,
 332 albeit being the most potent neutralizing antibodies against WT SARS-CoV-2.

333

334 By contrast, antibodies of the other three classes recognize evolutionarily conserved
 335 regions distinct from the RBM and some of these are often cross-reactive with other
 336 sarbecoviruses (65-67, 79). The binding of class IV antibodies, albeit attached to the
 337 apical shoulder of the RBM, is focused on a condensed patch that comprises residues
 338 345-346, 440-441, 444-446, 448-450, which are not related to mutations observed in

339 VOCs (Fig. 3C and fig. S16). Related to the binding position, site IV epitopes,
340 accessible in both “open” and “closed” conformations, exist either as partially
341 overlapped with or outside ACE2 binding sites (Fig. 3A). Interestingly, class IV
342 antibodies can execute their neutralizations via multiple mechanisms, such as (i)
343 direct blockage of RBD-ACE2 associations, (ii) bridging adjacent “closed” RBDs to
344 lock the S-trimer into a completely closed prefusion conformation, (iii) blockage of
345 viral membrane fusion by locking conformational changes of the S-trimer, or (iv) Fc-
346 dependent effector mechanisms (31, 62, 67). Class IV antibodies, e.g. 1-57, 2-7, S309
347 and BD-812, hold the greatest potential for harboring ultra-potent neutralization
348 activity and markedly high tolerance to most VOCs (63, 67). Not surprisingly, all class
349 IV antibodies, but CV07-270, exhibited excellent neutralizing breadth and potency to
350 VOCs (Fig. 3E). The probable reason underlying the exception could be that CV07-
351 270 bears an unusually long HCDR3, directly contacting E484, distal to the site IV (46).
352 Site V locates beneath the RBM ridge, opposite to the site I, and adjacent to the site
353 III. None of the class V antibodies compete with ACE2 binding (Fig. 3D and fig.
354 S17). Due to ~40% targeting frequency to L452, B.1.617.2, but not other VOCs,
355 partially decreased the activities of some class V antibodies (Fig. 3E). Class VI
356 antibodies recognize a patch on one side of the RBD, distal from the RBM. Among
357 these, some compete with ACE2 binding, while some do not, and this largely depends
358 on the orientation/pose of the antibodies bound. Both sites V and VI contain cryptic
359 epitopes that are only accessible when at least one RBD is in the open state (Fig. 3A
360 and C). In some cases, e.g. FC08 and CR3022, belonging to class V and VI,
361 respectively, epitopes are only accessible in the prefusion S-trimer under the
362 condition that all RBDs are open, suggesting that binding of these antibodies would
363 facilitate the destruction of the prefusion S-trimer (83, 84). In spite of less potency,
364 antibodies targeting sites V to VI are mostly tolerant to the VOCs.

365

366 Low levels of NAbs elicited by either natural infection or vaccination during *in vivo*
367 viral propagation may impose strong selection pressure for viral escape, leading to
368 an increase in the number of SARS-CoV-2 variants. To further understand the drivers
369 of viral evolution, we constructed immunogenic and mutational heatmaps for RBD

370 using the 171 NAb complex structures to estimate *in vivo* NAb-targeting frequencies
 371 on the RBD and viral mutation frequencies (calculated from the datasets in the
 372 GISAID), respectively (Fig. 3D and fig. S19). Briefly, for each antibody, we
 373 identified epitope residues and calculated the frequency of each RBD residue being
 374 recognized by antibody. Immunogenic heatmap revealed that the epitope residues of
 375 sites I to III showed predominantly higher NAb recognition frequencies (about 53.8,
 376 55.0 and 49.2 antibodies per residue on average for site I, II and III, respectively)
 377 compared with those of sites IV to VI (about 19.4, 9.1 and 14.3 antibodies per
 378 residues on average for site IV, V and VI, respectively), suggesting that class I to III
 379 antibody epitopes are “hot” immunogenic sites (Fig. 3D and fig. S19). In line with
 380 this, residues within sites I to III exhibited dramatically higher mutation frequencies,
 381 as revealed in circulating variants that include mutations of K417, L452, S477, T478,
 382 E484 and N501 residues (Fig. 3D and fig. S19). Surprisingly, none of the top 9 hottest
 383 immunogenic residues had a high mutation frequency. In particular, residues, such
 384 as F486, Y489, Q493, L455, F456, *et.al* (top 5, having 96, 96, 81, 73 and 70
 385 antibodies per residue, respectively) with large side chains exhibited extremely low
 386 mutation frequencies in circulating SARS-CoV-2 strains (Fig. 3D and fig. S20). It’s
 387 worthy to note that all these residues are extensively involved in the recognition of
 388 ACE2. The buried surface area (BSA) of these residues upon binding to ACE2
 389 confirmed that extensive interactions would be significantly reduced by amino acid
 390 substitutions, thereby affecting ACE2-mediated viral entry. Thus, genetic, structural
 391 and immunogenic analysis explains why mutations at these positions would not be
 392 selected.

393

394 A few studies have reported that a subset of NTD-targeting antibodies can be as
 395 potent as best-in-class RBD specific antibodies. They work *via* inhibiting a step post-
 396 attachment to cells like blocking fusion of the virus to the host cell membrane (85-
 397 88). We performed cluster analysis on 26 structures of the NTD-NAb complexes
 398 (including 2 structures solved in this manuscript) (fig. S21A) (54, 85-93). A
 399 dominant site α , defined as the “supersite” in more recent studies (85-88), comprising
 400 of three flexible loops (N1, N3 and N5), is the largest glycan-free surface of NTD

401 facing away from the viral membrane (facing up). Antibodies targeting site α
402 generally exhibited the most potent neutralizing activity compared to other sites on
403 the NTD (85, 90) (fig. S21B and C). The NTD supersite antibodies are primarily
404 derived from a subset of VH-genes with an over-representation of *IGHV1-24*. Sites
405 β and γ , as the left and right flank clusters, construct a shallow groove beneath the
406 supersite and locate at the back of the groove, eliciting less potent antibodies. By
407 contrast, δ antibodies, bound to a patch beneath the groove have their Fab constant
408 domains directed downward toward the virus membrane (facing down) (fig. S21B
409 and C). In line with binding orientation, many of the δ antibodies were shown to
410 present infection enhancing activities *in vitro* (54, 90). Perhaps correlated with being
411 a “hot” immunogenic site that is amenable to potent neutralization, highly frequent
412 mutations, including a number of deletions within the NTD supersite were identified
413 in most VOCs under ongoing selective pressure, leading to significant reduction and
414 in some cases even complete loss of neutralization activity for these NTD supersite
415 NAbs (94).

416

417 More recent studies have reported that SARS-CoV-2 infection can produce a long-
418 lasting memory compartment that continues to evolve over 12 months after infection
419 with ongoing accumulation of somatic mutations, emergence of new clones and
420 increasing affinity of antibodies to antigens (14, 15). Consequently, an increase in
421 breadth and overall potency of antibodies produced by memory B cells over time has
422 been revealed (14), akin to the experimental observations elicited by a 3-dose
423 vaccination strategy using an inactivated vaccine described in this study. To
424 investigate whether changes in the frequency of distribution of the six types of RBD
425 antibodies is associated with evolution time, we collated and categorized human
426 SARS-CoV-2 NAbs from available literatures. For antibody clustering, we combined
427 structural and square competition matrix analysis for 273 RBD NAbs in total (Fig.
428 4A and fig. S22). In the earliest documented studies (before Dec 2020), NAbs
429 belonging to classes I to III were predominantly identified in early COVID-19
430 convalescent and 2-dose vaccinated individuals (defined as early time point),
431 accounting for up to ~80% of total antibodies. By contrast, a low ratio of NAbs from

432 IV to VI was reported possibly due to their less potent activities at the early time
433 point (Fig. 4A). In recent literatures (after Dec 2020), NAbs with enhanced
434 neutralizing potency and breadth from IV to VI have substantially been enriched in
435 the late convalescents or 3-dose vaccinees, almost equal in frequency to antibodies
436 from I to III and further becoming ascendant in individuals immunized with 3 doses
437 of inactivated vaccine (Fig. 4A). Differential frequency of distribution of antibody
438 types may provide an additional possible explanation for the observed enhanced
439 neutralizing breadth of plasma in late convalescent individuals and 3-dose vaccinees.
440 These results suggest that memory B cells display clonal turnover after about 6
441 months, subsequently resulting in changes in the composition of antibodies in B cell
442 repertoire and thereby partially contributing to enhanced activities of antibodies
443 secreted in the plasma over time. To explore the underlying mechanism, we measured
444 the binding affinities of 167 type-classified antibodies that are also further
445 categorized into early and late time point groups (table S1 and fig. S9). For the late
446 time group, there was a 10-20 fold increase in binding affinity for individual classes,
447 compared to those in the early time point group (Fig. 4B). In early time point group,
448 antibodies from IV to VI exhibited higher binding affinities to the RBD than those
449 from I to III, in particular, antibodies from V and VI despite limited numbers (Fig.
450 4B). Possibly higher affinities for these antibodies are required to accomplish
451 neutralization successfully. Thus, most antibodies from V and VI with low affinities
452 and activities might be screened out in the early time point. In the late point group,
453 sub- nM binding affinities for individual class antibodies with no distinct variations
454 were observed, reflecting ongoing affinity maturation over time. This might also
455 explain the observation that some antibodies, from I to III isolated in the late time
456 point possess potent cross-neutralization activities (Fig. 3E). Our antibody clustering
457 and V gene usage analysis suggests that individual class antibodies can be derived
458 from multiple V genes and the shared V gene antibodies belong to different classes.
459 To decipher the intrinsic trends in the relationship between binding affinity and
460 somatic hypermutation (SHM) rate, we determined the relative affinity (K_D) and
461 calculated the SHM rate of antibodies that are encoded by the same V gene and
462 belong to the same class. The measured K_D -SHM plots and K_D -SHM log-log plots of

463 class I antibodies (n=61), including 32 NAbs derived from *IGHV3-53*, show least
464 squares fitting of data to a power law with a strong correlation of -0.81 for *IGHV3-53*
465 antibodies (-0.55 for all class I antibodies) (Fig. 4C). The absolute value of its slope
466 corresponding to a free energy change per logarithm (base e) *SHM* of cal nmol^{-1} ,
467 where free energy change is $4.98RT + 1.48RT \ln(\text{SHM})$ ($R = 2.0 \text{ cal K}^{-1} \text{ nmol}^{-1}$
468 and $T = 298 \text{ K}$). Antibodies with adequate numbers tested from II and III exhibited
469 similar trends by following a power law, among which *IGHV3-66* antibodies in class
470 II yielded a compelling correlation of -0.94 despite 6 plots involved in the fitting
471 (Fig. 4C). These trends indicate that as the SHM increase, the binding energy
472 increases and K_D value decreases.

473

474 More recently, the B.1.617.2 variant has contributed to another surge in COVID-19
475 cases worldwide, accounting for ~90% of new cases in the UK and >40% in the US,
476 despite the fact that increasing number of people have been vaccinated. Evaluation
477 of the effectiveness of several vaccines performed recently suggests that the efficacy
478 for VOCs correlates with full vaccination status and the time that has passed since
479 vaccination (95, 96). These may indicate that the effectiveness of the vaccines has
480 started to decline as months pass after vaccination due to fading immunity. Our
481 results demonstrate that a third-dose booster of inactivated vaccine can elicit an
482 expeditious, robust and long-lasting recall humoral response which continues to
483 evolve with ongoing accumulation of somatic mutations, emergence of new clones
484 and increasing affinities of antibodies to antigens, conferring enhanced neutralizing
485 potency and breadth. Collectively, our findings rationalize the use of 3-dose
486 vaccination regimens.

487

488

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503 all recombinant antigen proteins used in this manuscript. J-J.W., Y.H., L.W., J.L., X.G.,
504 Y-J.Z., H.Z. and B-S.L performed pseudovirus and authentic virus neutralization
505 experiments. K.W., L.W., P. L., WJ.F, N.W and L.Z performed structural study. Y.C.,
506 Y.Z., W.W. and Y.G. prepared PBMCs and flow cytometry sorting. Y.C. and A.Y.
507 performed 10X sequencing library construction. Z.J. and K.F performed BLI assay.
508 Y.H. and Q.G recruited volunteers and coordinated the collection of blood samples. All
509 authors analyzed data; X.W wrote the manuscript with input from all authors.
510 **Competing interests:** All authors have no competing interests. **Data and materials**
511 **availability:** Cryo-EM density maps of the SARS-CoV-2 S trimer in complex with
512 XGv013 or XGv043, the SARS-CoV-2 S trimer in complex with XGv004, XGv030
513 and XGv016; the SARS-CoV-2 S trimer in complex with XGv026 and XGv046, and
514 the SARS-CoV-2 S trimer in complex with XGv018, XGv038 and XGv42 have been
515 deposited at the Electron Microscopy Data Bank with accession codes EMD-UUUU,
516 EMD-WWWW, EMD-XXXX, EMD-YYYY and EMD-ZZZZ, respectively.

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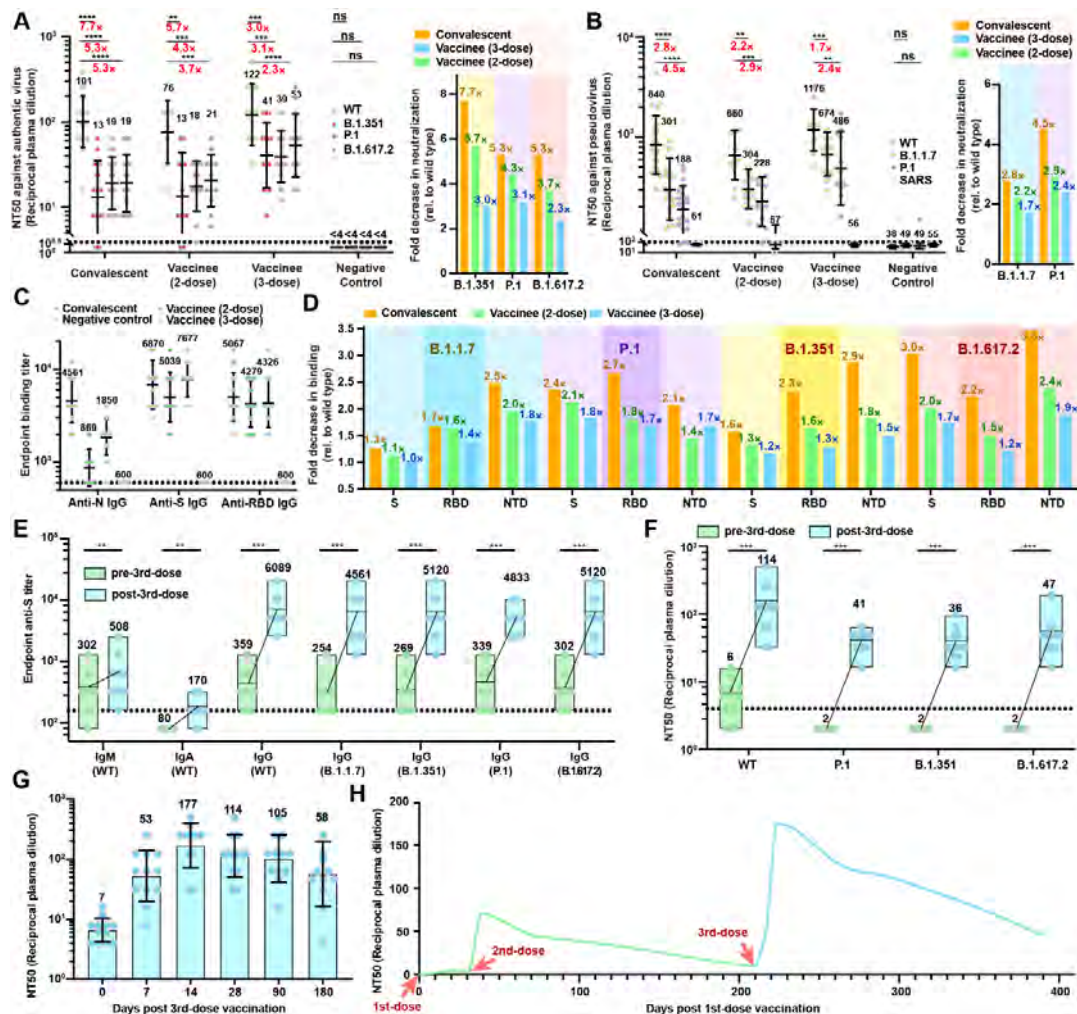
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525 **Figure legends**



526

527 **Fig. 1 A 3rd-dose booster of an inactivated vaccine elicits an expeditious and**
 528 **long-lasting recall antibody response**

529 Plasma neutralizing activity evaluated by authentic SARS-CoV-2 (A) and pseudo-
 530 typed SARS-CoV-2 neutralization assays (B) Left: half-maximal neutralizing titer
 531 (NT₅₀) values for plasma from COVID-19 convalescents, 2-dose, 3-dose CoronaVac
 532 vaccine recipients (at week 4 after the last dose of vaccination) and negative controls
 533 (pre-COVID-19 historical control) against live SARS-CoV-2 WT, B.1.351, P.1 and
 534 B.1.617.2, and VSV-based SARS-CoV-2 pseudoviruses bearing WT or B.1.1.7 or P.1
 535 S protein. Black bars and indicated values represent geometric mean NT₅₀ values.
 536 Statistical significance was determined using the two-tailed Wilcoxon matched-pairs
 537 test. Experiments were repeated in triplicate. Dotted lines indicate the limit of
 538 detection. Right: fold decrease in neutralization for each variant relative to WT for

539 each cohort of plasma samples (calculated from the left datasets) is shown.

540 **(C)** IgG endpoint antibody responses specific to the N, RBD and S of WT SARS-
541 CoV-2 were measured in plasma samples collected from cohorts as described earlier.

542 **(D)** Fold decrease in specific binding to the RBD, NTD and S for each variant over
543 WT for each cohort of plasma samples as described above.

544 **(E)** IgA, IgM and IgG endpoint antibody titers specific to the S of WT SARS-CoV-
545 2 or its variants in plasma samples collected from vaccinees before and 4 weeks after
546 the 3rd-dose immunization.

547 **(F)** Neutralizing titers against live SARS-CoV-2 WT, P.1, B.1.351 and B.1.617.2 for
548 plasma from vaccinees before and 4 weeks after the 3rd-dose immunization. Black
549 bars and indicated values represent geometric mean NT₅₀ values.

550 **(G)** Longitudinal neutralizing titers of plasma from 3-dose vaccinees at days 0, 7,
551 14, 28, 90 and 180 post the 3rd-dose vaccination. The geometric mean NT₅₀ values
552 are labeled.

553 **(H)** Kinetics of the 3rd-dose booster elicited recall response as indicated during
554 monitoring of NAb titers at different time points. The green and blue curves show
555 the changes in kinetics of NAb titers for pre-3rd-dose and post-3rd-dose vaccination,
556 respectively.

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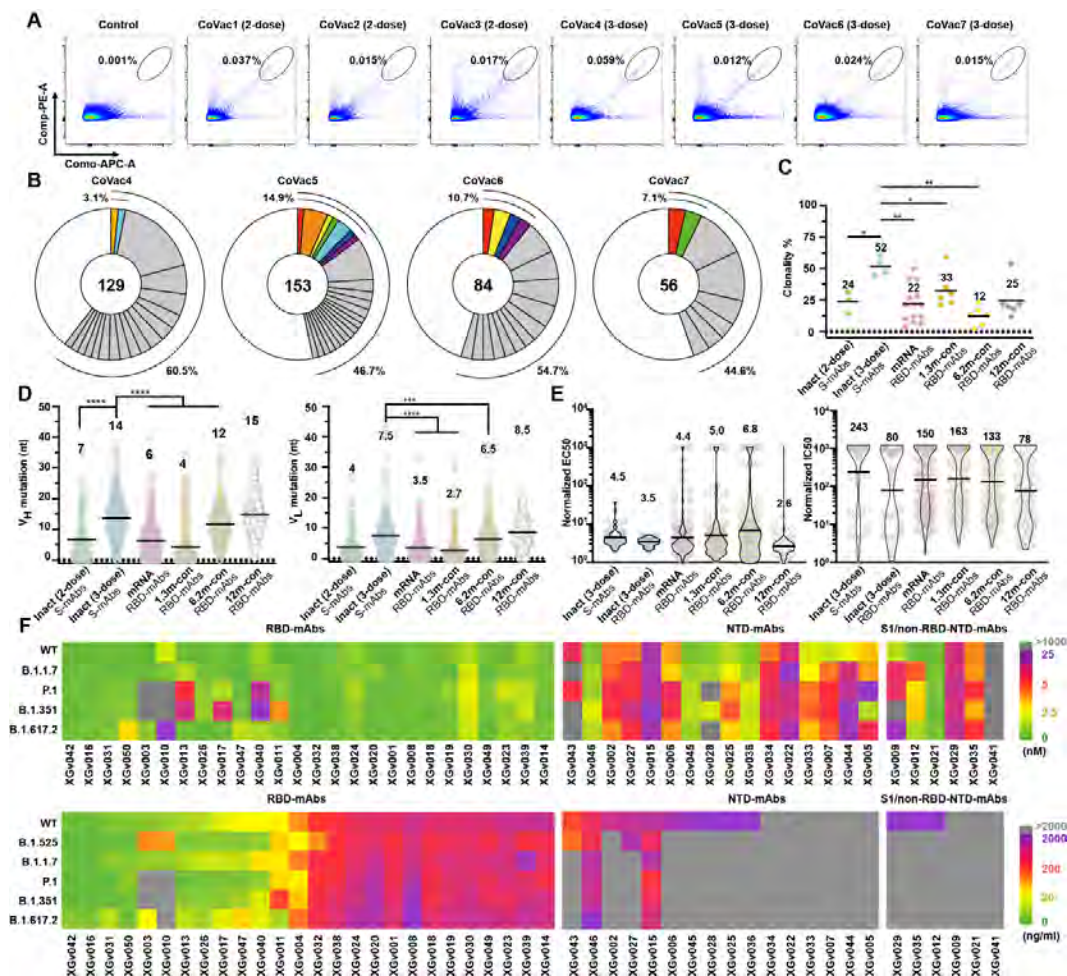
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569 **Fig. 2 Memory B cell antibodies elicited by a 3rd-dose booster of an inactivated**
 570 **vaccine**

571 (A) Representative flow cytometry plots showing dual allophycocyanin (APC)-S-
 572 and phycoerythrin (PE)-S-binding B cells for vaccinees and control donor.

573 (B) Pie charts represent the distribution of antibody sequences from the four 3-dose
 574 vaccinees. The number in the inner circle is the number of sequences analyzed here.
 575 Pie-slice size is proportional to the number of clonally related sequences. The black
 576 outline indicates the frequency of clonally expanded sequences detected individually.
 577 Colored slices reveal clones that share the same *IGHV* and *IGLV* genes.

578 (C) Graph shows relative clonality among seven individuals who received 2-dose or
 579 3-dose of inactivated vaccines. Relative clonality for COVID-19 convalescents
 580 assayed at 1.3, 6.2 and 12 months after infection, as well as 2-dose mRNA vaccine
 581 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).

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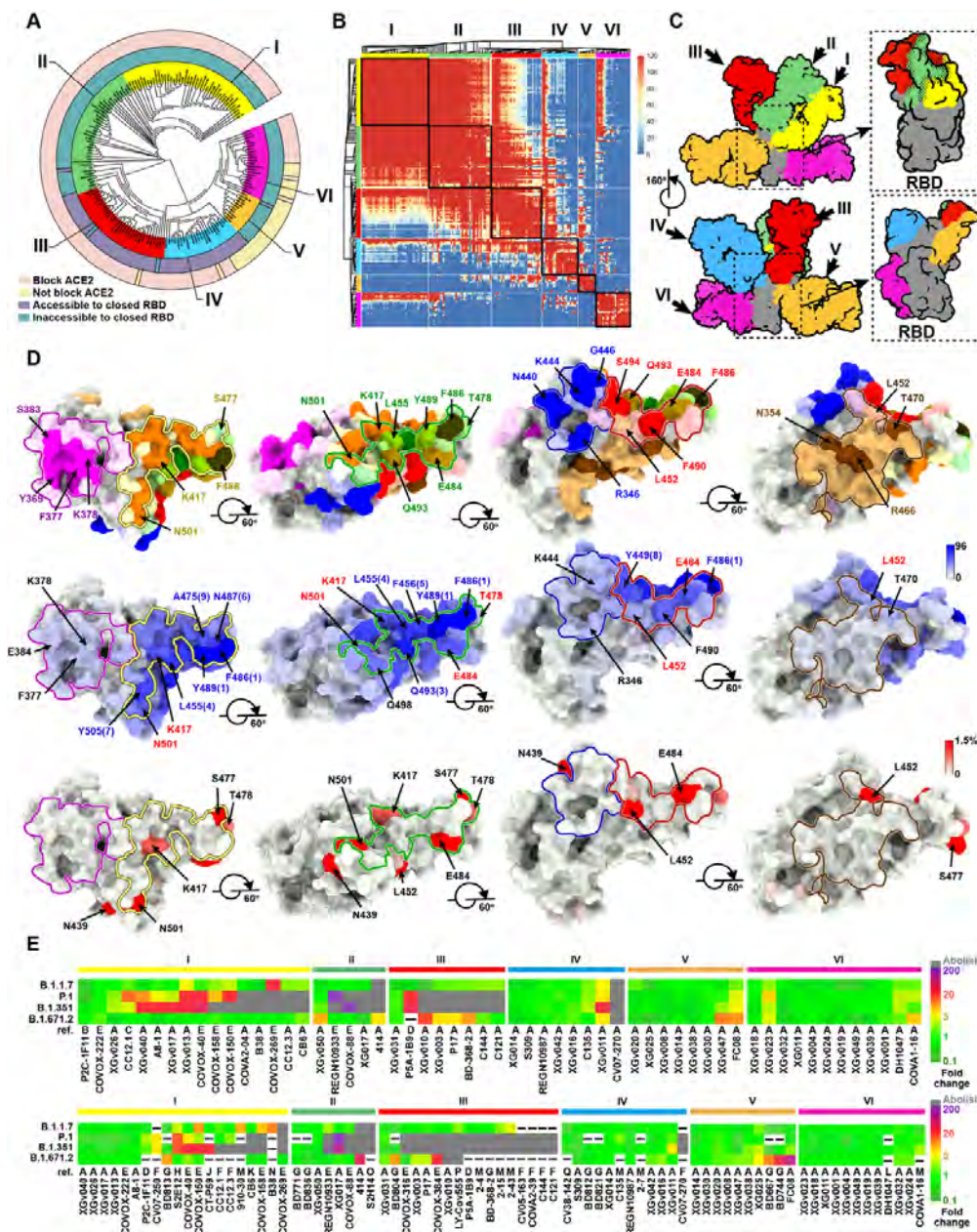
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611 **Fig. 3 Structural landscape and immunogenic features of RBD NAb**

612 (A) Structure-based antigenic clustering of SARS-CoV-2 RBD NAb. A total of 171
 613 RBD NAb with available structures were classified into six clusters (I, II, III, IV, V
 614 and VI). NAb that can block ACE2 binding or not are outlined by light pink and
 615 light yellow, respectively. NAb that can attach to the closed RBD or not are
 616 outlined by gray blue and gray green, respectively.

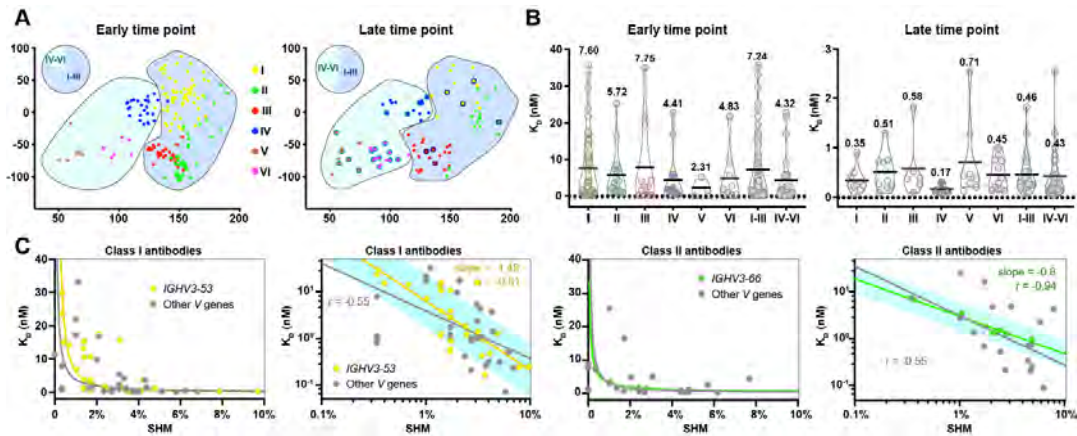
617 (B) Superimposition matrix of 171 RBD NAb structures' output from clashed areas
 618 (\AA^2) between variable regions of any two Fab fragments showing the clustering into

619 six antibody classes.

620 **(C)** Surface representative model of six types of NAbs bound to the RBD. Fab
 621 fragments of six representative antibodies are shown in different colors and the RBD
 622 is colored in gray. Insets illustrate the antigenic patches targeted by six representative
 623 antibodies. Dashed dots indicate the overlaps between two adjacent antigenic
 624 patches.

625 **(D)** Structural landscapes of the six classes of RBD NAbs (upper panel). Antigenic
 626 patches (with targeting frequency >30%) recognized by six classes of NAbs are
 627 outlined in the assigned color scheme (same to Fig. 3C), among which residues with
 628 “hot targeting frequency” (generally over 65%, but over 85% in class I) are shown
 629 in bright colors corresponding to the patches they belong to. Residues involved in
 630 two (such as Y489, L452) or three (such as F486) neighboring antigenic patches are
 631 presented in a mixed color. Representative “hot” antigenic residues are labeled.
 632 Middle: hot map for antigenic residues on the RBD. Per residue frequency
 633 recognized by the 171 NAbs were calculated and shown. The top 9 of the hottest
 634 antigenic residues and key residues with substitutions in several VOCs are marked
 635 and labeled. Bottom: hot map for circulating variants with mutations on the RBD.
 636 Mutation frequency for each residue was calculated based on the datasets from
 637 GISAID.

638 **(E)** Immunogenic characteristics of six classes of RBD-targeting NAbs. Hot maps
 639 show relative fold changes in K_D values (up) and IC_{50} values (down) against several
 640 VOCs for the six classes of NAbs, including previously reported (97-108) and newly
 641 isolated antibodies described in this manuscript. Color gradients for upper and
 642 bottom panels indicate relative fold changes and are shown at right side. “-”: no
 643 related datasets in the original studies and related references are listed. Ref “A”
 644 indicates that the datasets were produced in this manuscript. Other letters in Ref
 645 correspond to different reference numbers shown as below. B – 91 and this
 646 manuscript, C – 99 and this manuscript, D – 97, E – 30, 81, 103 and 104, F – 99, G –
 647 98, H – 100 and 108, J – 101, K – 94 and 102, L – 105 and 106, M – 94, N – 105, O
 648 – 107, P – 82, Q – 66, respectively.



649

650 **Fig. 4 Antibody evolution and affinity maturation**

651 (A) Uniform manifold approximation and projection (UMAP) plot displaying the
 652 antibodies defined as the early time point group (left) and late time point group
 653 (right). The antibodies are colored based on their cluster assignments by the
 654 hierarchical clustering algorithm. Antibodies from I to III and IV to VI are
 655 highlighted in cyan and gray blue background, respectively. Pie charts represent the
 656 frequency distribution of antibodies belonging to I to III and IV to VI. Antibodies
 657 isolated from 3-dose vaccinees are outlined by black lines.

658 (B) Dissociation constants (K_D) of the antibodies from I to VI. Individual class
 659 antibodies are represented in colors corresponding to the classes they belong to. The
 660 color scheme is same as Fig. 4A. BLI traces are shown in fig. S9.

661 (C) The measured K_D -SHM plots (left) and K_D -SHM log-log plots (right) of
 662 antibodies from I and II are shown. *IGHV3-53* and *IGHV3-66* antibodies belonging
 663 to class I and II are colored in yellow and green, respectively. The straight curves
 664 and lines are the least squares fits of the data to the power law with the values of the
 665 slope for *IGHV3-53* and *IGHV3-66* antibodies. The black curves and lines indicate
 666 the fitting of antibodies from I or II; the yellow and green ones suggest the fitting of
 667 *IGHV3-53* and *IGHV3-66* antibodies, respectively. The cyan lines are the 90%
 668 predicted interval.

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CoronaVac

What science proves

2.9. Countries that chose inactivated virus vaccines, such as CoronaVac, are more protected against SARS-CoV-2 variants, says Spanish study

A study conducted by researchers at the University of Barcelona, in Spain, concluded that vaccines against Covid-19 prepared with inactivated virus, as is the case of CoronaVac, the vaccine of Butantan and the Chinese pharmaceutical company Sinovac, confer greater effectiveness in the medium and long term in controlling the pandemic, compared to immunizers made with other technologies, due to their performance against variants of the SARS-CoV-2 virus.

According to Joan Serrano-Marín and Rafael Franco, authors of the article “Two urgent needs in the battle against COVID-19: a classic-type vaccine and specific medication,” published in the OSF preprints platform, new vaccine technologies developed at an emergency pace to combat the pandemic, such as messenger RNA and adenovirus viral vector, can confer high protection against the original SARS-CoV-2 strain, but tend to lose efficacy as new variants emerge.

“Classical vaccines, such as CoronaVac, promote the generation of a broader repertoire of antibodies and cellular responses. In other words, they allow us to neutralize the virus using more diverse strategies. An example is the positive situation experienced in countries like Chile, China and Uruguay, where the main vaccine used has been the CoronaVac”, explain Joan and Rafael in an exclusive interview for the Butantan Portal.

Inactivated virus immunizers contain all the parts of the dead virus. This may generate a broader immune response than those of messenger RNA vaccines or vaccines that use adenovirus as the viral vector, since they use only a part of the Spike protein (used by SARS-CoV-2 to infect cells).

The article suggests that reinfection and collapse of health systems can occur in countries that use the messenger RNA or adenovirus vaccines, even though the percentage of the population vaccinated is high – just as happened in Israel. The same trend, that is, new pandemic waves after mass vaccination with RNA/adenovirus vaccines, would be seen, according to the researchers, in several European countries and in the United States.

“The viral load of the delta variant is very high for vaccinated and unvaccinated. In other words, the vaccinated will continue to infect the vaccinated and unvaccinated. Herd immunity, in general terms, is achieved when the average number of infected infects less than one person per infected. By way of explanation, transmission must be drastically reduced. As the calculations indicate, for the same percentage of vaccinated people, the transmission is extremely lower in countries that have used CoronaVac as the main vaccine” Joan and Rafael add.

Performance of inactivated virus vaccine

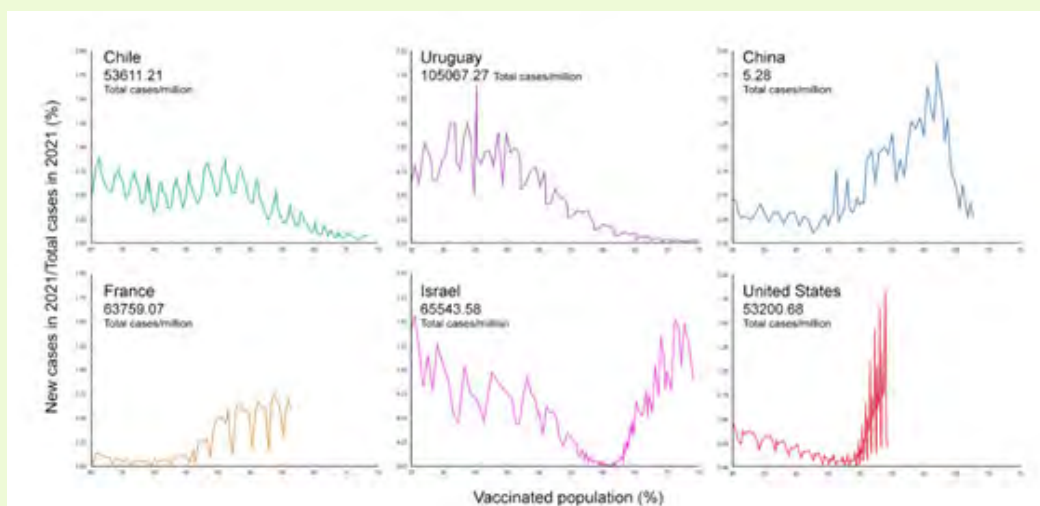
Countries such as the United States, Israel and the United Kingdom have faced an upsurge in the number of Covid-19 cases, despite high vaccination rates. The reason is the arrival of the delta variant (B.1.617.2, Indian), more transmissible. This is an opposite trend to that seen in Chile, Uruguay and China, which have used Coronavac as the main immunizer.

In the cases of Uruguay and Chile, the increase in the percentage of the population vaccinated with CoronaVac led to a considerable reduction in the proportion of new cases. As for China, the scientists point out that neither the increases nor the decreases

are significant, because the total of 2,021 cases, measured per million, is insignificant in comparison with the other countries (five new cases per million inhabitants in China, compared to 65,543 in Israel or 53,200 in the United States).

According to the researchers, administration of CoronaVac and other inactivated virus immunizers is highly desirable for achieving herd immunity because of the broad spectrum of antibodies they generate in vaccinated individuals, including a greater diversity and amount of neutralizing and non-neutralizing antibodies, and their greater ability to respond to possible mutations or genetic drift of all SARS-CoV-2 proteins.

“The greater number of immune strategies that traditional vaccines induce is mainly



due to the fact that starting from the complete virus, in the case of CoronaVac, the immune system is able to induce a greater repertoire of responses, making this process more effective. This is not the case with modern vaccines, such as messenger RNA or adenovirus, all of them are designed to focus on a single protein of the coronavirus, the S protein, which can also mutate when the virus mutates,” Joan and Rafael summarize.

How inactivated virus vaccines work

Each dose of inactivated virus vaccines, whose technology has been known for more than a century, is composed of trillions of particles of the virus in question. As they are inactivated, these particles are incapable of causing the disease in those who receive the immunizer. Their function is another: to stimulate the immune system to recognize the virus as soon as it comes into contact with it.

As CoronaVac contains the entire inactivated SARS-CoV 2 virus, the

immune system produces antibodies that recognize many antigens (proteins) of the new coronavirus. The S protein is the main one, used by SARS-CoV-2 to penetrate human cells, but not the only one. The coronavirus has a total of 29 proteins, most of them are responsible for regulating the multiplication and exit of the virus from human cells. Thus, a variant that has an alteration in the S protein (mutation) is no longer recognized by specific vaccines containing only the S protein.

Modern vaccines are designed to give the immune system the ability to identify the S protein, stimulating the production of neutralizing antibodies, which are our body’s main weapons in fighting the virus. Traditional vaccines, such as CoronaVac, as they contain the whole virus, are able to stimulate the immune system to recognize all the proteins to a greater or lesser extent, triggering the production of both neutralizing antibodies to the S protein, as well as several others related to other proteins in the viral arsenal.

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Two urgent needs in the battle against COVID-19: a classic-type vaccine and specific medication

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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

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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](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

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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 things 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

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25). In sharp contrast, France, Israel and the US shows an increase of new cases upon increased vaccination using mRNA/AV vaccines.

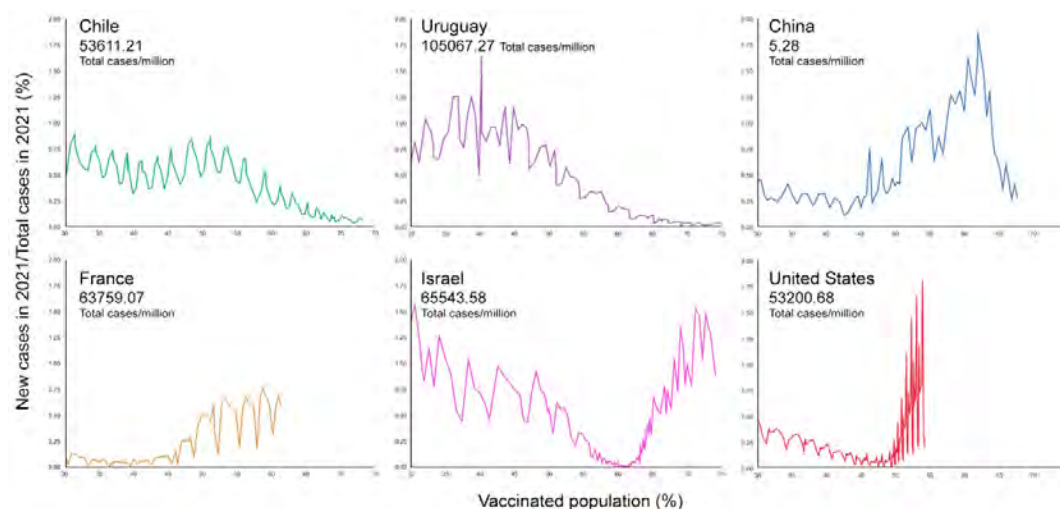


Figure 1. New COVID-19 cases versus percentage of vaccinated population. Data (retrieved until August 24, 2021) have been selected using 30% vaccinated population as threshold. Chile, Uruguay and China have mainly used CoronaVac vaccine. France, Israel and the US have used only mRNA/AV vaccines. The 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

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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).

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CoronaVac

What science proves

3 ■ It is safe for pregnant and babies

3.1. CoronaVac has efficacy of 85% in the prevention of severe cases of Covid-19 on pregnant, shows study

A research made by Brazilian and British scientists showed that CoronaVac, the vaccine from Butantan and the Chinese pharmaceutical Sinovac, had the efficacy of 85% to prevent severe cases of Covid-19 among Brazilian pregnant. The study was published in the preprint platform SSRN, which is linked to the magazine The Lancet, and its authors are from the London School of Hygiene and Tropical Medicine, the Federal University of Bahia, the Oswaldo Cruz Foundation, the University of Brasília and the State University of Rio de Janeiro.

According to the researchers, the efficacy of the complete immunization scheme with two doses of CoronaVac was 85% to prevent severe cases of Covid-19, and 75% in the prevention of the progression of symptomatic cases to the severe form of the disease. No deaths occurred among the pregnant that were partially or completely immunized with CoronaVac, while four deaths were expected if the mortality rate was the same of the non-vaccinated public.

The population studied was of all the pregnant with Covid-19 symptoms, between 18 and 49 years of age,

with registered PCR tests realized between 15/03 and 3/10 of 2021, and registered in the System of Notification from the Health Ministry (e-SUS Notifica). At the end of the triage were selected the data of 19.838 pregnant, being 7.424 (37,4%) with positive tests for Covid-19, and 588 (7,9%) developed the severe form of the disease. At the moment of the extraction of the data, 83% of the pregnant had received both doses of the vaccine, while 17% had received only one dose.

“A complete use of CoronaVac on pregnant was efficient in the prevention of symptomatic cases of Covid-19 and with a high efficacy in the prevention of the severe form of the disease”, emphasized the researchers.

In 17/01 of 2021, the Health Ministry began the vaccination against Covid-19 with CoronaVac. In 15/03, pregnant women with comorbidities and in occupations considered of high risk became eligible to receive the vaccine. On 26/04, the recommendation of the immunization was expanded to include all the pregnant.

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Effectiveness of the CoronaVac vaccine in prevention of symptomatic and progression to severe Covid-19 in pregnant women in Brazil

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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.

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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 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 biologically 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.

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Figure 1: Flowchart of the study population from surveillance system and final sample of cases and controls

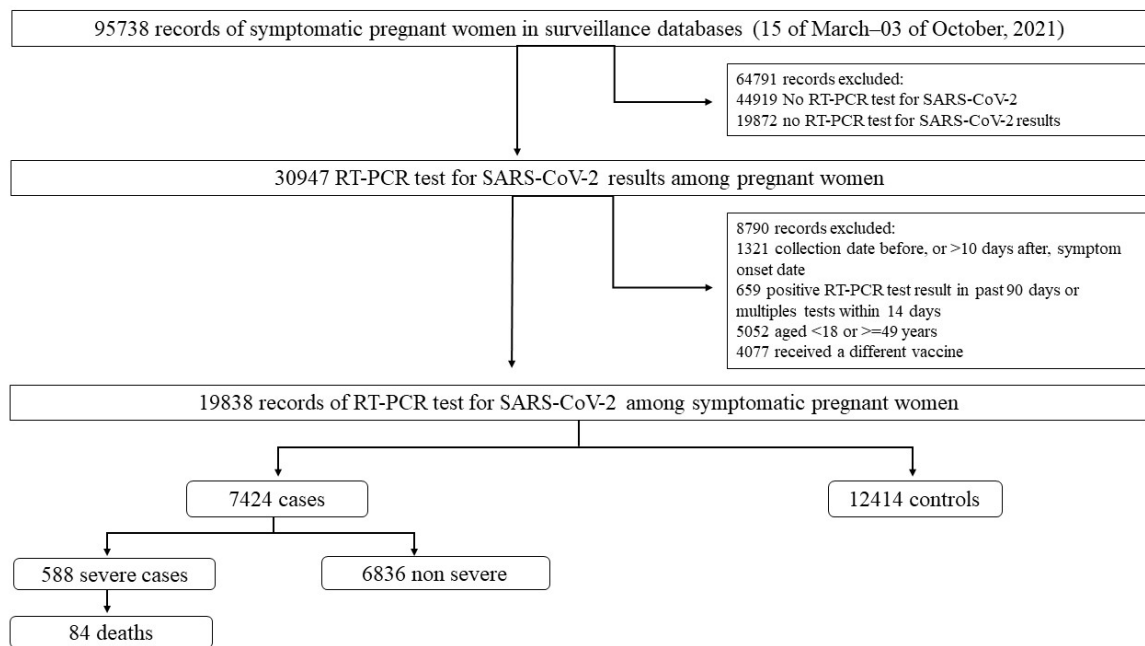


Figure 2: Number of cases and controls by interval since first and second vaccination

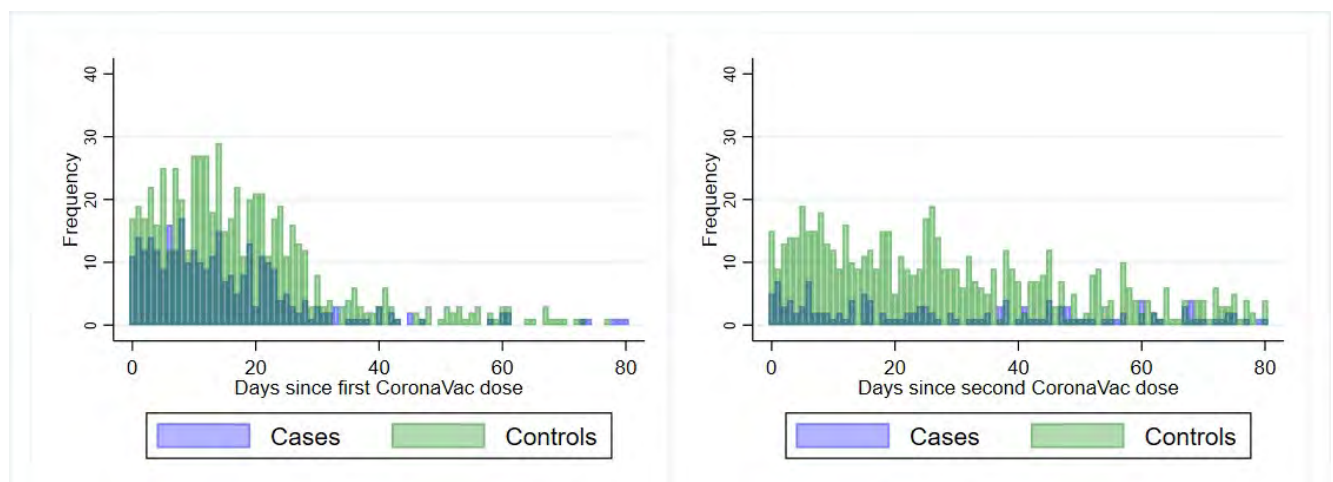


Table 1: Characteristics of cases and controls in pregnant women aged 18-49 years in Brazil.

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 N° 1/2021-DAPE/SAPS/MS - Vaccination for pregnant and postpartum women with comorbidities	<ul style="list-style-type: none"> - 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 N° 467/2021-CGPNI/DEIDT/SVS/MS - Vaccination for pregnant and postpartum women without comorbidities	<ul style="list-style-type: none"> 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 n° 627/2021-CGPNI/DEIDT/SVS/MS - Temporary suspension of vaccination	<ul style="list-style-type: none"> - Temporary suspension of vaccination with the vaccine AstraZeneca/Oxford/Fiocruz in pregnant and postpartum women
19/05/2021	NOTA TÉCNICA N° 651/2021 - CGPNI/DEIDT/SVS/MS - Continued vaccination in pregnant and postpartum women with comorbidities	<ul style="list-style-type: none"> - 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 N° 2/2021 - SECOVID/GAB/SECOVID/MS - Continued vaccination in pregnant and postpartum women without comorbidities	<ul style="list-style-type: none"> - 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 N° 6/2021-SECOVID/GAB/SECOVID/MS - Interchangeability between vaccines for pregnant and postpartum women who	<ul style="list-style-type: none"> - 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	<ul style="list-style-type: none">- 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
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3.2. Protection against Covid-19 generated by CoronaVac is transmitted to the babies through breast milk, demonstrates research

A study made by the Clinical Hospital of the Medicine School from University of São Paulo (HCFMUSP) points that lactating women that received the CoronaVac, vaccine produced by Instituto Butantan in partnership with the Chinese pharmaceutical Sinovac, presented antibodies against Covid-19 in the breast milk, capable of protecting the babies as well, up to four months after the vaccination.

The research was realized with 20 employees that were immunized between January and February of 2021. There were collected a total of nine samples of breast milk: before the immunization, four times after the first dose and three times after the second dose, with gaps of seven days and four months after the vaccination.

The research showed that the levels of antibodies of the breast milk were still high four months after the vaccination. The peak of the production of antibodies happened in the second week after the first

dose and in the fifth and sixth week after the second dose.

The immunization of breastfeeding and pregnant offers protection in two ways: to the babies that are still not born, through the placenta, with IgG antibodies, and through the breast milk, to the newborn, with IgA antibodies.

According to the Health Ministry, around 500 thousand pregnant and those who recently gave birth that have comorbidities were already vaccinated against Covid-19 in Brazil. The pregnant began the priority group in the vaccination campaign since the lethality rate of Covid-19 among them is much higher than the average (10% for pregnant and 2% for the general population). Only two vaccines are recommended for pregnant, CoronaVac being one of them, for having a high efficacy and high profile of safety.

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CoronaVac can induce the production of anti-SARS-CoV-2 IgA antibodies in human milk

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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 (17th-21st) and February (15th-18th), 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 antiseptic 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 -20°C 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).

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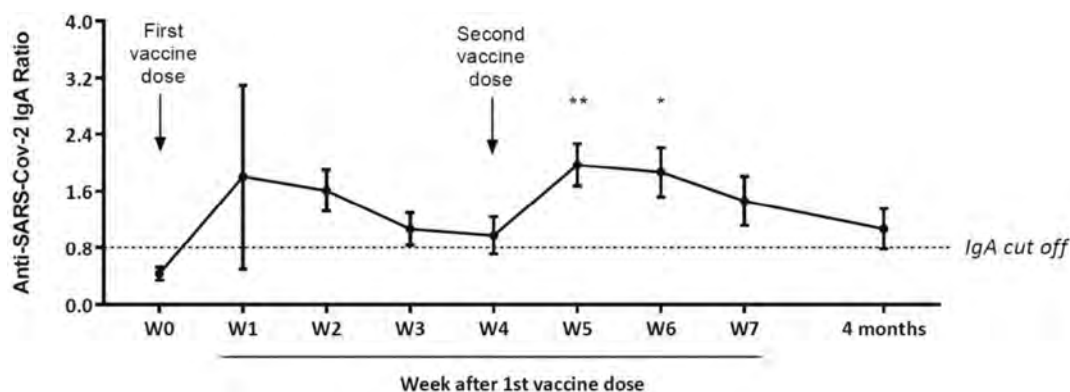


Figure 1 - Anti-SARS-CoV-2-specific IgA ratios (mean ± standard error) in milk samples collected over time (Weekly-W) from 16 healthy mothers previously COVID-negative after a 2-dose schedule of the CoronaVac vaccine (Sinovac Biotech Ltd., China). The last withdrawal was performed four months after the 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).

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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.

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CoronaVac

What science proves

4. It protects individuals with comorbidities

4.1. CoronaVac is safe and effective in patients at increased risk for thrombosis, study says

Research conducted in São Paulo with individuals at high risk of thrombosis, with an autoimmune disease called antiphospholipid syndrome, showed that CoronaVac is a safe and effective vaccine for this population. The work was published in the journal *Lupus* by scientists from the School of Medicine of the University of São Paulo (FMUSP) and coordinated by the researcher and rheumatologist Eloisa Bonfá, clinical director of the Hospital das Clínicas at FMUSP.

Recent international reports of thrombosis in people who had received adenovirus vaccines have caught the eye of the scientific community. To assess this risk in the case of CoronaVac, an inactivated virus vaccine, the researchers selected 44 patients with antiphospholipid syndrome and 132 controls, with a mean age of 46 years. All participants received two doses of CoronaVac.

Regarding the amount of antiphospholipid antibodies in patients with the syndrome, no significant difference was found between samples collected before and after vaccination, showing that

CoronaVac is safe and does not influence the disease. The volunteers were followed for six months and no thrombotic events were observed during this period.

“No thrombotic event was recorded during our study. 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”, the researchers state.

The vaccine also proved to be highly immunogenic. Six weeks after the second dose, both groups showed comparable high seroconversion, with IgG antibody production in 83.9% of patients and 93.5% of controls. Patients also showed high positivity for neutralizing antibodies (77.4%), with activity of 64.3%. No serious or moderate adverse effects were observed.

According to the scientists, the safety and immunogenicity findings support the CoronaVac recommendation for patients with antiphospholipid syndrome.

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Immunogenicity, safety, and antiphospholipid antibodies after SARS-CoV-2 vaccine in patients with primary antiphospholipid syndrome

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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 ($\alpha\beta 2$ GPI) 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

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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 β 2GPI, 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). Sero-conversion 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

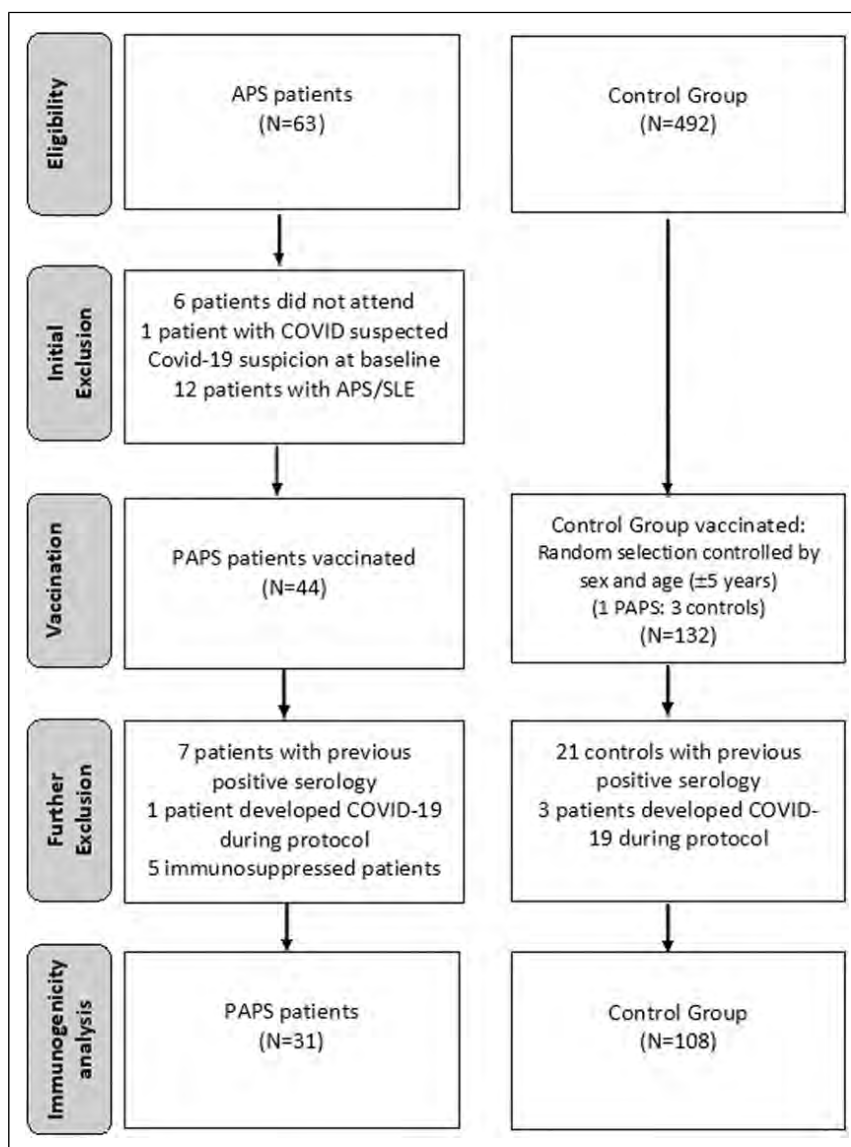


Figure 1. Flowchart of patients and controls submitted to Sinovac-CoronaVac vaccination.

Table 2. Seroconversion rates and anti-SARS-CoV-2 S1/S2 IgG titers before and after CoronaVac in naïve-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.

^ap<0.001 for longitudinal comparisons of GMT in PAPS patients at D28 and D69 vs. baseline.

^bp<0.001 for longitudinal comparison of GMT in PAPS patients at D69 vs. D28.

^cp<0.001 for longitudinal comparison of GMT in control at D28 and D69 vs. baseline.

^dp<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 naïve-PAPS compared to controls.

	After vaccine 1 st dose		After vaccine 2 nd 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).

^ap <0.05 in comparison to controls.

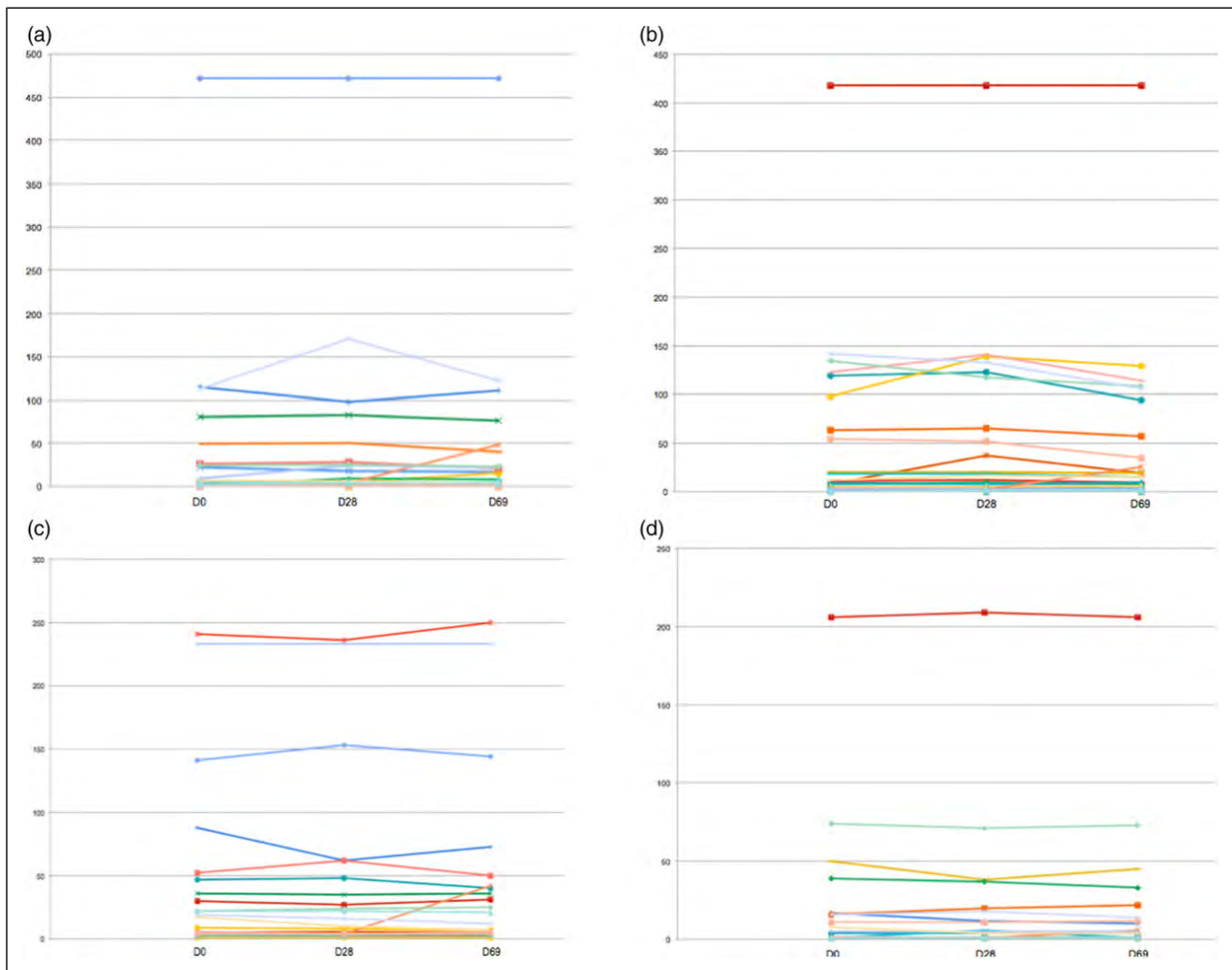


Figure 2. Antiphospholipid antibody titers evaluation in naïve primary antiphospholipid patients before (baseline—D0) and after Sinovac-CoronaVac vaccination (first dose—D28 and second dose—D69). (a) Anticardiolipin antibody IgM (aCL, titers in MPL), (b) anticardiolipin antibody IgG (aCL, titers in GPL), (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 1 st dose			After vaccine 2 nd 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 features³⁴ 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 (BBIBPCoV, 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.

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Declaration of conflicting interests

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Trial registration

ClinicalTrials.gov- NCT04754698 first registered on February 8th 2021. <https://clinicaltrials.gov/ct2/show/NCT04754698?term=NCT04754698&draw=2&rank=1>

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4.2. CoronaVac is safe for cancer patients, Hong Kong study shows

A study carried out in Hong Kong with 74,000 patients with cancer or with a history of the disease once again showed that CoronaVac is a safe vaccine for this population, with the lowest rate of adverse reactions compared to messenger RNA vaccines. The paper was published in the Journal of Hematology & Oncology and conducted by researchers at the School of Medicine of the University of Hong Kong.

We selected 74,878 individuals who had already been diagnosed with cancer, divided into two groups: patients with active cancer (25,789 or 34% of the total) and people with a history of cancer (49,089 or 66%). Volunteers received two doses of CoronaVac or Pfizer and the results were compared to those of unvaccinated patients.

Those who took CoronaVac had the lowest incidence of adverse reactions recorded in the entire trial. In patients with active cancer, the incidence of daily adverse events was 0.13 per 10,000 people vaccinated with CoronaVac, and 0.31 in those who received a vaccine produced with messenger RNA technology. Among the unvaccinated, the incidence was higher, at 1.02.

Among people with a history of cancer, the occurrence of daily adverse effects from CoronaVac was 0.42 per 10,000 people, while that of the messenger RNA vaccine was 0.55. In the group that did not receive any immunization, the rate was 0.93.

The researchers concluded that none of the vaccines increases

the frequency of adverse events in patients with cancer or with a history of the disease – on the contrary, those who are not vaccinated are more at risk of suffering from these events. During the study period in September 2021, the overall vaccination rate against Covid-19 in Hong Kong reached 58.8%, but only 30.2% of cancer patients had been vaccinated.

“The findings of this study can reassure clinicians 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”, the scientists point out.

Adverse Event of Special Interest

During the pandemic, the World Health Organization (WHO) defined that, in the case of Covid-19 immunization campaigns, an adverse event of special interest (AESI) is a specific clinically significant event associated with a vaccine, the effects of which need to be carefully monitored and evaluated.

According to the WHO, conditions commonly identified as AESI include Guillain-Barré syndrome, acute encephalomyelitis, anaphylaxis, serious events potentially related to new vaccines and adjuvants, as well as any disease whose increased severity is associated with the immunizing agent, such as cancer.














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BRIEF REPORT

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Safety of two-dose COVID-19 vaccination (BNT162b2 and CoronaVac) in adults with cancer: a territory-wide cohort study

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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

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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

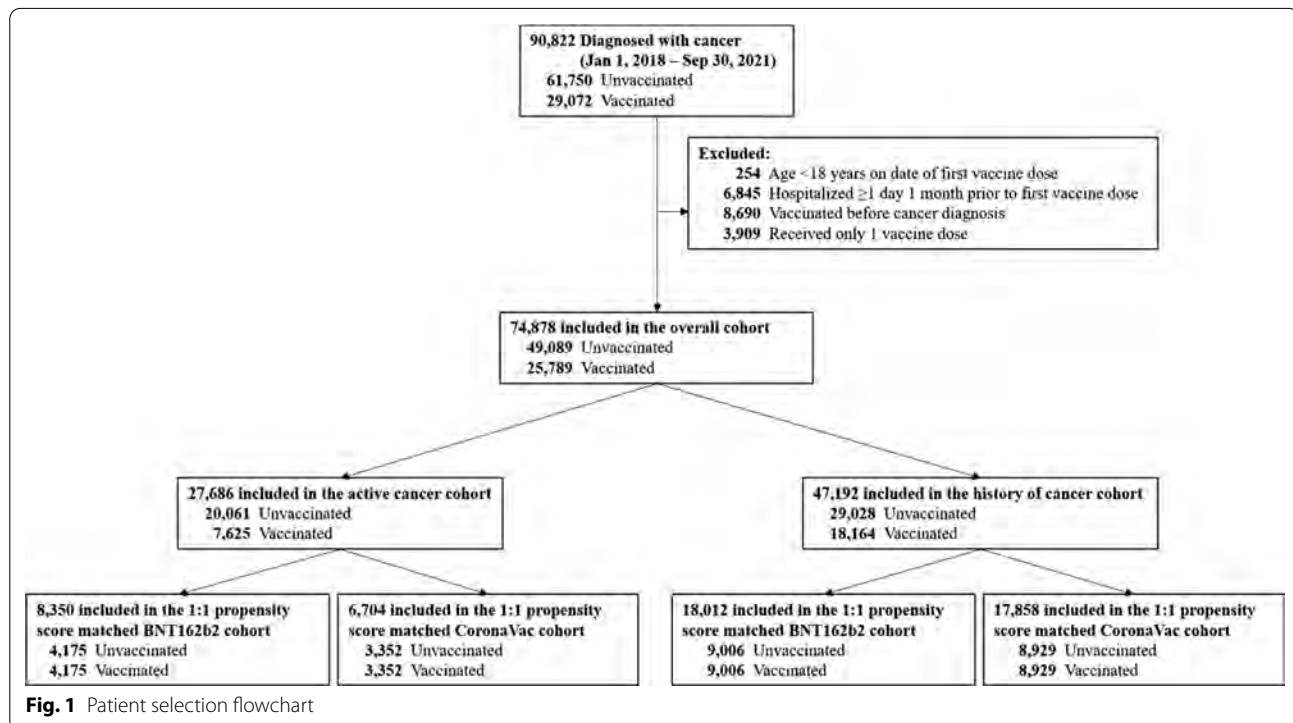
vaccine dose for patients who were vaccinated with either BNT162b2 or CoronaVac. For unvaccinated patients, the pseudo index date was selected from a corresponding vaccine recipient matched on age and sex. Patients younger than 18 years, hospitalized within 30 days before vaccination, or diagnosed with 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



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)		
<i>Active cancer</i>								
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)		
<i>History of cancer</i>								
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.

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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.

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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.

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CoronaVac

What science proves

4.3. Third dose of CoronaVac promotes robust immune response in patients with rheumatic diseases

The third dose of CoronaVac, a vaccine from Butantan and Chinese pharmaceutical company Sinovac, induces a high immune response against Covid-19 in patients with autoimmune rheumatic diseases, with antibody production in more than 90% of individuals. This is the main finding of a study carried out at the Hospital das Clínicas, School of Medicine of the University of São Paulo, published in the Annals of the Rheumatic Diseases of the British Medical Journal.

The scientists included 597 adult patients with autoimmune rheumatic diseases in the study, as well as a control group of 199 healthy people, with comparable mean age and gender distribution in both groups. All participants had been vaccinated with two doses of CoronaVac and received the third dose six months after the second. Patients with controlled disease discontinued immunosuppressive medications during the vaccination period.

Among patients, the seroconversion rate of IgG antibodies against SARS-CoV-2 increased significantly with the third dose, from 60% to 93% one month after application. Likewise, the positivity of neutralizing antibodies increased from 38% before the third dose to 81.4% after 30 days of the booster dose. The same pattern was observed for the control group.

The third dose of CoronaVac also boosted the immune response of patients who were not producing

antibodies six months after the second dose. With the booster, seroconversion reached 80.5% for IgG antibodies and 59.1% for neutralizing antibodies.

Importantly, the drop in seroconversion six months after the second dose has already been proven by science in all Covid-19 vaccines currently in use, and is related to the dynamics of the SARS-CoV-2 virus, not the efficacy of the immunizing agents.




Additional analyses revealed that factors such as advanced age, vasculitis, and use of prednisone (corticosteroid) and mycophenolate mofetil (to prevent transplant rejection) were associated with reduced IgG antibody positivity. In addition, the use of prednisone, abatacept, belimumab and rituximab were related to lower production of neutralizing antibodies.

According to the researchers, the study reinforces the importance of the third dose for people with autoimmune rheumatic diseases. “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.”

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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

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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.

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(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 variant⁵⁻⁷ 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 dose¹⁷ 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 disease³¹⁻⁴⁰ 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

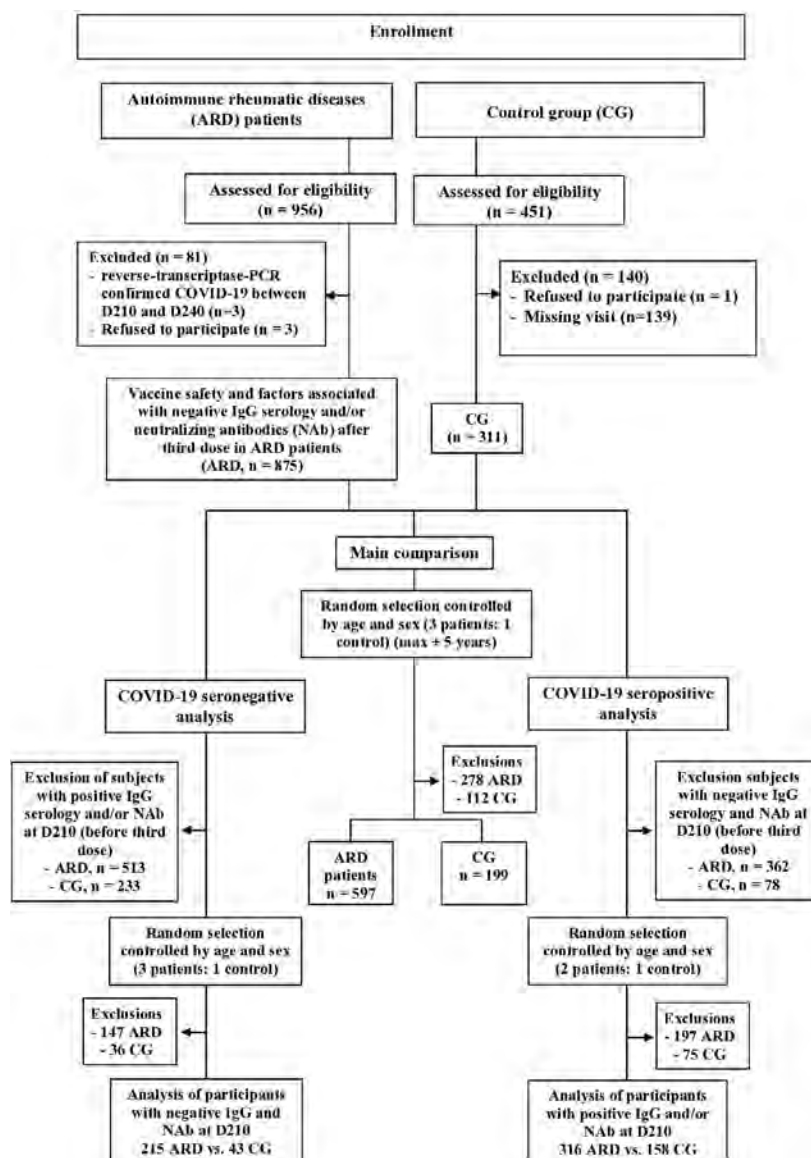


Figure 1 Flow chart diagram. The diagram shows the flow of eligible individuals, exclusions and analysed participants. Reasons for exclusions are given in the figure. D210, 6 months after second dose.

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

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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			
Methotrexate	172 (28.8)	–	–
Leflunomide	71 (11.9)	–	–
Mycophenolate mofetil	73 (12.2)	–	–
Azathioprine	62 (10.4)	–	–
Others*	33 (5.6)	–	–
Biologic agent			
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

Groups	Anti-S1/S2 IgG positivity			NAb positivity		
	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 $\geq 30\%$ (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

Groups	Anti-S1/S2 IgG GMT (95% CI), AU/mL			Neutralising activity, median % (IQR)		
	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 CDC⁴³ 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

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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+)

Groups	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)		
	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 $\geq 30\%$ (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.

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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.

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CoronaVac

What science proves

4.4. CoronaVac induces higher antibody production in people with chronic hepatitis B, Chinese study says

A study published in the journal *Liver International* showed that CoronaVac, a vaccine from Butantan and Sinovac, is highly effective in patients with chronic hepatitis B, inducing greater antibody production in this population than in healthy individuals. The work was conducted by scientists at Chongqing Medical University in China. Chronic hepatitis is an inflammation of the liver caused by the hepatitis B virus that lasts for more than six months and increases the risk of getting liver cancer.

The study included 362 adult patients with chronic hepatitis (48 with cirrhosis and 73 with active viral replication) and 87 healthy individuals in the control group, all vaccinated with two doses of inactivated virus vaccines, CoronaVac or the Sinopharm immunizing agent.

The IgG antibody seroconversion rate was similar between the two groups after one, two and three months of the second dose. The antibody titer was lower in patients in the first month, but from the second month onwards, this value was similar to that of healthy individuals. “Interestingly, at 3 months, titers of anti-spike IgG, anti-RBD IgG and RBD-ACE2 blocking antibody were all higher in CHB patients than in controls,” the authors state.

The researchers assessed anti-Spike, anti-RBD and RBD-ACE2 blocker IgG antibody titers. When comparing

patients and controls, the results showed a titer of 161.6 vs 85.9; 275.4 vs 132.5 and 4.7 vs 3.2, respectively.

In addition, volunteers with chronic hepatitis showed a slower decline in antibodies over time than people without the disease. There was also no difference in the immune response between patients with and without cirrhosis and with and without active viral replication.

All adverse effects reported were mild and the incidence of reactions was similar between patients and controls (14% and 11.5%, respectively). The most common symptoms were pain at the injection site and fatigue.

CoronaVac is safe for patients with liver disease

A number of studies have already attested to the safety and efficacy of the Butantan vaccine for people with liver problems. A Chinese study showed that the vaccine induced high seroconversion rates in patients with hepatitis B, being 87.25% for IgG antibodies and 74.5% for neutralizing antibodies. Another study published on The Lancet’s SSRN preprint platform demonstrated 100% production of IgG antibodies in subjects with metabolic-associated fatty liver disease (MAFLD).

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Safety and antibody response to inactivated COVID-19 vaccine in patients with chronic hepatitis B virus infection

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Abbreviations:

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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.

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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.

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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.

Accepted Article

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 population¹⁻³. Currently, there are about 292 million people worldwide infected with HBV, and 86 million in China alone⁴.

Vaccination is an effective intervention in preventing SARS-CoV-2 infection, severe symptom and death⁵⁻⁷. 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 immunity¹¹⁻¹³. 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 guideline¹⁴. 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 SARS-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 °C 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 μ g/mL human ACE2 recombinant protein (100 μ L per well) by incubation at 4 $^{\circ}$ C overnight. After thoroughly discarding solutions in the plate, 300 μ L 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 μ L) and RBD protein linked to HRP (RBD-HRP) (50 μ L) 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 $100 - [(OD \text{ value of sample} / OD \text{ value of RBD-HRP only control}) \times 100\%]$. According to the manufacturer's instructions, a positive result for the SARS-CoV-2 blocking antibody

was determined when the inhibition rate was $\geq 20\%$. 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°C 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°C 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 ($10^{-4.93} \times 10^7$ 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 ($p \geq 0.05$) (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 cirrhosis⁹.

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 vaccination⁹. 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 diseases¹⁵⁻¹⁷. A previous study has shown that atypical memory B cell is a short-lived activated cell that may represent a precursor plasma cell (PC) population¹⁸. 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 ALT¹⁹ 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-infection²¹. Previous studies have shown that SARS-CoV-2-specific memory B cells can be retained in an infected individual for at least 5-6 months²²⁻²⁴, or even as long as 1 year²⁵. 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.

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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%)	0.857
24-28, n (%)	32 (36.8%)	122 (33.7%)	
≥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

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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)	<i>P</i> 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

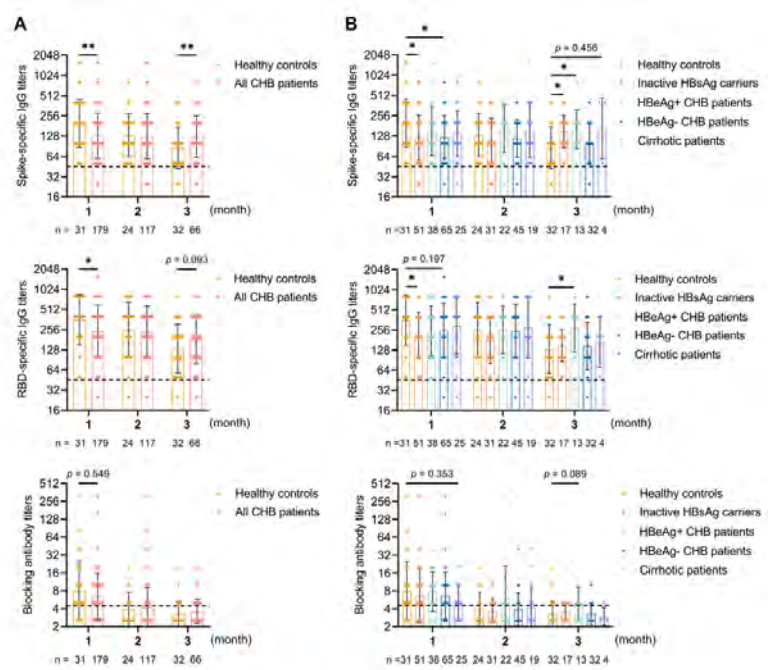
Chi-square test and Fisher's exact test were used to compare statistical difference of seropositivity rates of antibodies at 1, 2 and 3 months after full-course vaccination between healthy controls and CHB patients. RBD, receptor binding domain. $p < 0.05$ was considered statistically significant.

Figure legends

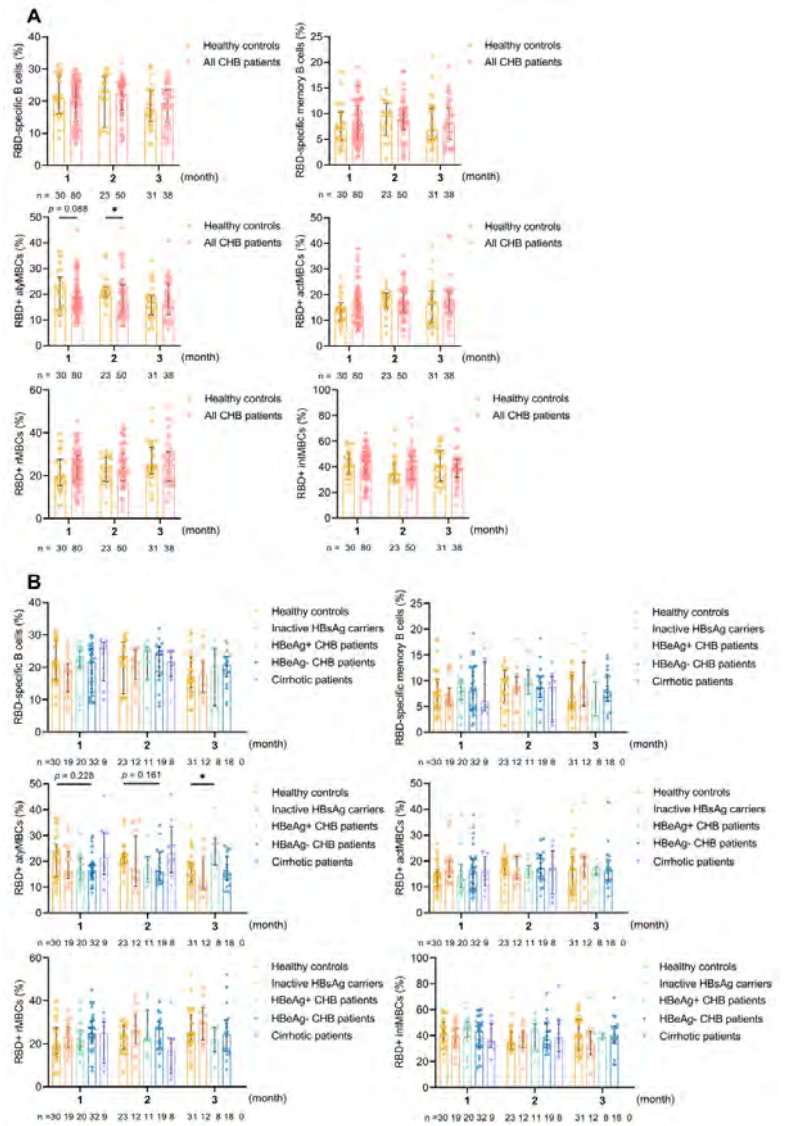
Figure 1 Antibody response after full-course vaccination in CHB patients and healthy controls. **(A)** Titers of spike-specific IgG, RBD-specific IgG, blocking antibody in healthy controls (n = 87) and all CHB patients (n = 362) at 1, 2 and 3 months after full-course vaccination. **(B)** Titers of spike-specific 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

4.5. CoronaVac induces antibodies in 85,2% of the patients with cancer, demonstrates Turkish study

A study published in the journal Future Oncology demonstrated that CoronaVac, vaccine from Butantan and the chinese pharmaceutic Sinovac, has efficacy in the protection of people with cancer, inducing the production of high titers of antibodies in 85,2% of the analyzed patients. The work was conducted by Turkish researchers from the Bezmialem Vakif University, Medipol University, Okmeydani Hospital of Research and Training, Ankara Hospital, among other institutions.

The scientists evaluated the seropositivity of CoronaVac on 776 patients with cancer, adults with an average age of 64, that entered the oncologic clinic between 01/03 and 01/07 of 2021. The control group was composed of 715 people that were not diagnosed with cancer, with an average age of 50 years. All were vaccinated with two doses, with a gap from four to six weeks.

Among the patients, 85,2% produced antibodies against SARS-CoV-2, with an average titer of 363,9 UA/mL. In the control group, the tax of seropositivity was of 97,5% and the average titer of antibodies was of 656,5 UA/mL.

The incidence of adverse effects after the first dose was 15,9% in the group of patients and 22,5% in the control group, being the most reported symptom fatigue and pain in the area of the injection. In relation to the second dose, there was no significant difference in the adverse reactions.

The tumoral types more common were breast cancer (32,3%), lung cancer (23,6%), gastrointestinal cancer (22,4%) and genitourinary cancer (13,8%). From the patients, 51,3% (398 people) presented metastatic disease; 39,8% (309 people) were in active

chemotherapy; 15,1% (117 people) were in immunotherapy or targeted therapies; and 45,1% (350 people) did not receive any of these modalities of treatment in the previous three months.

According to the researchers, the significant factors associated with the smaller taxes of seropositivity in the group of the patients were age and active chemotherapy. However, the results confirmed the efficacy and safety of CoronaVac in this population.

Differences of seropositivity among the patients

To compare the taxes of antibodies production among the patients, the scientists divided the participants into four subgroups: group of active chemotherapy, immunotherapy group, targeted therapies group and hormonal therapy group.

The tax of seropositivity was 78,6% on the active chemotherapy group, 85,7% in the immunotherapy group, 86% in the targeted therapies group and 87,1% in the hormonal therapy group. For the patients that did not receive any treatment, the tax of seropositivity was 91,1%. Besides, 90,7% of the patients without metastasis and 79,9% of the patients with metastasis developed antibodies.

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Research Article

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Efficacy and safety profile of COVID-19 vaccine in cancer patients: a prospective, multicenter cohort study

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Aim: To compare the seropositivity rate of cancer patients with non-cancer controls after inactive SARS-CoV-2 vaccination (CoronaVac) and evaluate the factors affecting seropositivity. **Method:** Spike IgG antibodies against SARS-CoV-2 were measured in blood samples of 776 cancer patients and 715 non-cancer volunteers. An IgG level ≥ 50 AU/ml is accepted as seropositive. **Results:** The seropositivity rate was 85.2% in the patient group and 97.5% in the control group. The seropositivity rate and antibody levels were significantly lower in the patient group ($p < 0.001$). Age and chemotherapy were associated with lower seropositivity in cancer patients ($p < 0.001$). **Conclusion:** This study highlighted the efficacy and safety of the inactivated vaccine in cancer patients.

Clinical Trials Registration: NCT04771559 (ClinicalTrials.gov)

Plain language summary: Cancer patients are at high risk for infection with SARS-CoV-2 and of developing the associated disease, COVID-19, which therefore puts them in the priority group for vaccination. This study evaluated the efficacy and safety of CoronaVac, an inactivated virus vaccine, in cancer patients. The immune response rate, defined as seropositivity, was 85.2% in the cancer patient group and 97.5% in the control group. The levels of antibodies, which are blood markers of immune response to the vaccine, were also significantly lower in the patient group, especially in those older than 60 years and receiving chemotherapy. These results highlight the importance of determining the effective vaccine type and dose in cancer patients to protect them from COVID-19 without disrupting their cancer treatment.

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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

oncology clinics between 1 March and 1 July 2021 were informed about the study; the control group consisted of healthcare workers and volunteers accompanying the patients. From this initial group, 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. Vaccination information and the COVID-19 history of the participants were checked from the national health record database. Patients and controls who had a documented COVID-19 infection (positive PCR test result) at any time before enrollment and patients who received an mRNA vaccine were excluded. In addition, controls who were pregnant or had an immunosuppressive disease or were receiving immunosuppressive therapy for any reason were excluded from the study. The study was carried out with permission of the Turkish Ministry of Health and approved by the local ethics committee (02/28). All participants signed a written informed consent form.

Assessments

Blood samples were taken from the patients and centrifuged at 2500 rpm for 10 min. The separated serum samples were backed up in two Eppendorf tubes and stored at -80 or -20°C. All serum samples were delivered by cold chain and collected in a single center. A US FDA-approved chemiluminescent microparticle immunoassay, the Abbott Architect i1000sr SARS-CoV-2 IgG II Quant assay (Abbott Laboratories, IL, USA), was used to quantify IgG antibodies against the SARS-CoV-2 spike receptor-binding domain following the manufacturer's instructions [22]. This assay has 98.1% sensitivity and 99.6% specificity at least 15 days after first symptom onset or documented COVID-19 infection [23]. An IgG level ≥ 50 AU/ml is accepted as seropositive.

Patient characteristics were collected and included age, sex, BMI, smoking status, comorbidities and receipt of any other vaccination (influenza or pneumococci) within 2 years. All participants were asked about local and systemic side effects of vaccination. Additionally, all clinical information about the cancer diagnosis (tumor type, disease stage and treatment status) were recorded. Treatment groups were: chemotherapy group (including taxane, platin, fluorouracil, gemcitabine, anthracycline, cyclophosphamide, pemetrexed); immunotherapy group (including nivolumab, pembrolizumab and atezolizumab); targeted therapies group (tyrosine kinase inhibitors, anti-VEGF agents, trastuzumab, pertuzumab, CDK4/6 inhibitors); and hormonal therapies group (tamoxifen, aromatase inhibitors, LHRH analogs). We evaluated each treatment group for seropositivity. Additionally, we created another group for those receiving active targeted or immunotherapies and compared the seropositivity rates of this group with those of the active chemotherapy group.

Statistical analysis

Descriptive statistics are shown as mean \pm standard deviation for variables with normal distribution, median (minimum to maximum) for non-normal distributions, and the number of cases and percentage (%) for nominal variables. The Mann–Whitney U-test was used for comparison of the groups. Pearson's χ -square or Fisher's exact tests were performed for nominal variables. Multivariate analysis was applied with a logistic regression test. A p-value < 0.05 was considered to be statistically significant. SPSS for Windows (v. 22; IBM Corp., NY, USA) was used to analyze the data.

Results

Our study group consisted of 776 cancer patients and 715 non-cancer controls. The median age in the patient group was 64 years (range: 20–88), and the median age in the control group was 50 years (range: 21–94). The characteristics of the study participants are shown in Table 1.

The seropositivity rate was 85.2% and the median antibody titer was 363.9 AU/ml in the patient group. The seropositivity rate was 97.5% and the median antibody titer was 656.5 AU/ml in the control group. When the two groups were compared, the seropositivity rate and antibody levels were significantly lower in the patient group than in the non-cancer controls ($p < 0.001$). Additionally, administration of influenza and pneumococcal vaccine prevalence 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 22.5% in the control group. The rate of side effects reported after the first dose was significantly higher in the controls than the patients ($p = 0.001$). While the most common side effect in the control group was local pain (9.7%), the most common side effect in the patient group was fatigue (6.4%). When the prevalence of side effects after the second dose was compared, there was no significant difference between the two groups (Table 3).

The most common tumor types were breast cancer (32.3%), lung cancer (23.6%), gastrointestinal cancer (22.4%) and 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.

Characteristics	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 (%)	
First dose	15.9				22.5				0.001†
Local pain	5.7	5.3	0.4	-	9.7	8.3	1.1	0.3	0.005†
Erythema	0.5	0.4	0.1	-	2.1	1.8	0.3	-	0.009†
Fever	2.1	1.2	0.8	0.1	1.8	1.7	-	0.1	0.852
Fatigue	6.4	5.0	1.3	0.1	8.4	6.7	1.4	0.3	0.165
Headache	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
Nausea	1.8	1.7	-	0.1	1.4	1.4	-	-	0.681
Diarrhea	0.8	0.4	0.4	-	1.0	0.8	0.2	-	0.783
Other	0.9	0.9	-	-	2.6	2.6	-	-	0.269
Second dose	15.2				16.8				0.436
Local pain	5.0	4.6	0.4	-	7.7	6.2	1.3	0.3	0.042†
Fever	1.3	1.0	0.3	-	2.1	2.0	0.1	-	0.234
Fatigue	6.7	4.9	1.5	0.3	6.4	5.1	1.0	0.3	0.917
Headache	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
Nausea	1.2	0.8	0.4	-	0.7	0.7	-	-	0.427
Diarrhea	0.9	0.7	0.1	0.1	0.3	0.3	-	-	0.182
Other	1.1	1.1	-	-	1.3	1.3	-	-	0.647

†Statistically significant results.
Gr: Grade.

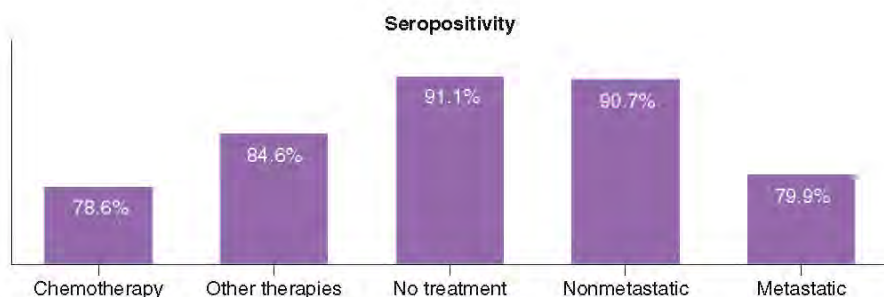


Figure 1. Seropositivity rates of cancer patients after SARS-CoV-2 vaccination according to the treatment status and stage of the disease.

on active chemotherapy; 15.1% (n = 117) were on immunotherapy or targeted therapies; and 45.1% (n = 350) had not received any of these treatment modalities within the previous 3 months. The seropositivity rates were 78.6% in the active chemotherapy group, 85.7% in the immunotherapy group, 86.0% in the targeted therapies group and 87.1% in the hormone therapy group. For the patients not receiving any active treatment including chemotherapy, immunotherapy or targeted therapies, the seropositivity rate was 91.1% (Table 4). Additionally, 90.7% 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 correlated with seropositivity (p < 0.001). The seropositivity rate in the active chemotherapy group was significantly lower than in the group of patients not receiving active chemotherapy (p < 0.001). Tumor type, BMI, smoking and comorbidities were not associated with seropositivity (Table 4). In univariate analysis of the control group, age was found to be the only factor negatively correlated with seropositivity (p < 0.001; Table 4). When the multivariate analysis was performed, age and chemotherapy were defined as the factors significantly associated with lower 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)			
Characteristics	n (%)	Seropositivity (%)	p-value
Age (years)			<0.001†
<60	291 (37.5)	93.5	
≥60	485 (62.5)	80.2	
Gender			0.015†
Female	433 (55.8)	88.0	
Male	343 (44.2)	81.6	
BMI			0.435
<25 kg/m ²	187 (30.7)	88.8	
≥25 kg/m ²	422 (69.3)	86.3	
Smoking			0.577
No	436 (59.2)	85.3	
Ex-smoker	165 (22.4)	87.3	
Yes	112 (17.8)	83.0	
Tumor 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	
Treatment 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	
Chemotherapy			<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	
Immunotherapy (IO)			0.920
Yes	42 (5.4)	85.7	
No	734 (94.6)	85.1	
Targeted therapies			0.811
Yes	178 (22.9)	86.0	
No	598 (77.1)	84.9	
Hormone therapy			0.426
Yes	209 (26.9)	87.1	
No	567 (73.1)	84.5	
Comorbidities			0.225
No	373 (48.1)	86.9	
Yes	403 (51.9)	83.6	
Stage			<0.001†
Nonmetastatic	378 (48.7)	90.7	
Metastatic	398 (51.3)	79.9	
Factors affecting seropositivity in the control group (univariate analysis)			
Characteristics	n (%)	Seropositivity (%)	p-value
Age (years)			<0.001†
<60	614 (85.9)	98.4	
≥60	101 (14.1)	92.1	
Gender			0.347
Female	398 (55.7)	98.0	
Male	317 (44.3)	96.8	
BMI			0.435
<25 kg/m ²	118 (30.5)	97.5	
≥25 kg/m ²	269 (69.5)	97.0	
Smoking			0.711
No	428 (72.2)	97.2	
Ex-smoker	16 (2.7)	93.8	
Yes	149 (25.1)	97.3	
Comorbidities			0.399
No	544 (76.1)	97.8	
Yes	171 (23.9)	96.5	

†Statistically significant results.
IO: Immunotherapy.

Table 5. The factors affecting seropositivity in the study population (multivariate analysis).

Characteristics	SE	RR	95% CI	p-value
Noncancer vs cancer	0.286	3.519	2.009-6.162	<0.001†
Age (<60 vs ≥60)	0.246	3.545	2.190-5.737	<0.001†
Gender (female vs male)	0.194	1.271	0.868-1.859	0.218
Comorbidities (yes vs no)	0.195	1.129	0.771-1.655	0.533

†Statistically significant results.
RR: Relative risk; SE: Standard error.

Table 6. The factors affecting seropositivity in the patient group (multivariate analysis).

Characteristics	SE	RR	95% CI	p-value
Age (<60 vs ≥60)	0.276	3.016	1.758-5.176	<0.001†
Gender (female vs male)	0.221	1.154	0.701-1.667	0.724
Chemotherapy (yes vs no)	0.358	1.396	0.692-2.818	0.038†
Targeted therapy or IO (yes vs no)	0.300	0.709	0.393-1.277	0.351
Comorbidities (yes vs no)	0.213	1.116	0.736-1.692	0.606
Stage (metastatic vs nonmetastatic)	0.304	1.458	0.804-2.645	0.214

†Statistically significant results.
IO: Immunotherapy; RR: Relative risk; SE: Standard error.

Discussion

This study showed 85.2% seropositivity in cancer patients, whereas this rate was 97.5% in non-cancer controls. Additionally, IgG antibody titers in cancer patients were significantly lower than in the controls. The factors significantly associated with low seropositivity rates in the patient group were age and active chemotherapy. When the side effects in both groups were compared, the control group reported significantly more side effects after the first dose. Nevertheless, there was no significant difference between the groups in side effects after the second dose. Our 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 in cancer patients, covidophobia, delays in cancer diagnosis and disruptions to oncological treatments increased the mortality of cancer patients during the pandemic [4-11]. NCCN and other oncological societies recommended that all cancer patients, especially those receiving active treatment, should be vaccinated as a priority [15]. The high seropositivity rate of cancer patients in our study also supports these recommendations, even though the seropositivity 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 expected, as immunosuppression negatively affects the immune response. Similar to our results, Ariamanesh *et al.* found that older age, chemotherapy and hematological malignancies were related to lower seropositivity rates after administration of inactivated vaccine [20]. Massarweh *et al.* reported that chemotherapy plus immunotherapy treatment was associated with lower IgG titers in cancer patients vaccinated with the BNT162b2 mRNA vaccine [21]. Furthermore, studies evaluating the response to pneumococcal and influenza vaccines in patients with malignancy showed a decreased response in patients with hematological malignancies [24]. In another study, influenza vaccine response was low in breast cancer patients receiving active chemotherapy [25]. Our findings also highlight the negative effect of active treatment on immune response.

Although a clear relationship has not yet been established between antibody levels and prevention of the disease, the main target of the vaccines is to trigger the formation of neutralizing antibodies against the SARS-CoV-2 spike protein [26]. Harvey *et al.* reported an approximately tenfold increase in positive nucleic acid amplification test results among patients with positive antibody tests compared with those who had negative antibody tests, suggesting a protective effect of antibodies [27]. Another study demonstrated that the antibody titers were correlated with protection against COVID-19 [28]. Considering that the cellular immune response is suppressed in cancer patients, even adequate antibody levels may not effectively protect from the infection. Based on this, the application of additional doses, especially in cancer patients, may come to the fore in light of future studies. Patients receiving active 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 the first dose. The reason might be that cancer patients experience such side effects due to the disease itself and their treatment processes, even before vaccination. The frequency of side effects reported after the second dose was found to be similar in both groups; this can be explained by the decrease in the perception of the side effects following the second dose.

Finally, when we created two groups by matching the patient and control groups by age and gender, the significant 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 not assess neutralizing antibody levels. However, studies have shown that neutralizing antibody levels are correlated with spike IgG antibody levels [29]. Second, we did not evaluate the pre-vaccination antibody levels of the participants. 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-19 during this period. The patient group will be followed up for long-term results to evaluate the effect of vaccination and antibody levels on disease prevention.

Conclusion

This study highlighted the efficacy and safety of CoronaVac in cancer patients. The seropositivity rate was lower in cancer patients than in non-cancer controls, especially in patients aged over 60 years and those receiving active chemotherapy. Further studies with larger sample sizes are needed to determine the effective vaccine type and vaccine dose for cancer patients so that cancer patients might be protected from COVID-19-related morbidity and 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 administration 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 protection 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 principles 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.

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4.6. Patients with autoimmune rheumatic diseases that already contracted Covid-19 may be protected with only one dose of CoronaVac, suggests study

A study published in The Lancet Rheumatology demonstrated that a single dose of CoronaVac, vaccine from Butantan and Sinovac, can be enough to promote an immune response in patients with autoimmune rheumatic diseases that were previously infected with SARS-CoV-2. The research was conducted in the Clinical Hospital of the Medicine School from São Paulo University (FMUSP).

According to the research, 95% of the 157 patients that had already contracted Covid-19 and were immunized with CoronaVac produced an expressive average of IgG antibodies after the first dose. After the second dose, the indicator jumped to 98% of the volunteers. The researchers also analyzed 471 individuals with rheumatic diseases that never had contact with the coronavirus. The complete immunization of both doses of the vaccine on that group induced the production of antibodies in 75% of the participants.

Also participated in the study 1.193 patients and 492 controls. After the random selection of samples, 942 people were analyzed (157 with positive serology and 471 with

negative serology). Both groups also counted, each one, with 157 individuals from control.

The researchers collected blood samples from the volunteers immediately before the first dose (day zero), before the second dose (day 28) and 69 days after the first dose (or 40 days after the second dose)

Immunological memory

The results of the article from USP support other researches made with individuals with autoimmune rheumatic diseases that had positive and negative serology, that shows that vaccines with messenger RNA and adenovirus induce the same pattern of immune response observed in the study with CoronaVac. "A possible mechanism that explains that robust response on those that already contracted Covid-19 is related to the memory B cells pre-existent, because the recurrent exposure is known for generating responses more extensive than of a primary infection", pointed the authors of the article.

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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



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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.

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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 diseases⁴ 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, spondyloarthritis, 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 AJS 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 ($\geq 30\%$ 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 timepoints. 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 sampling. We assessed incident case surveillance in all participants of CoronavRheum 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

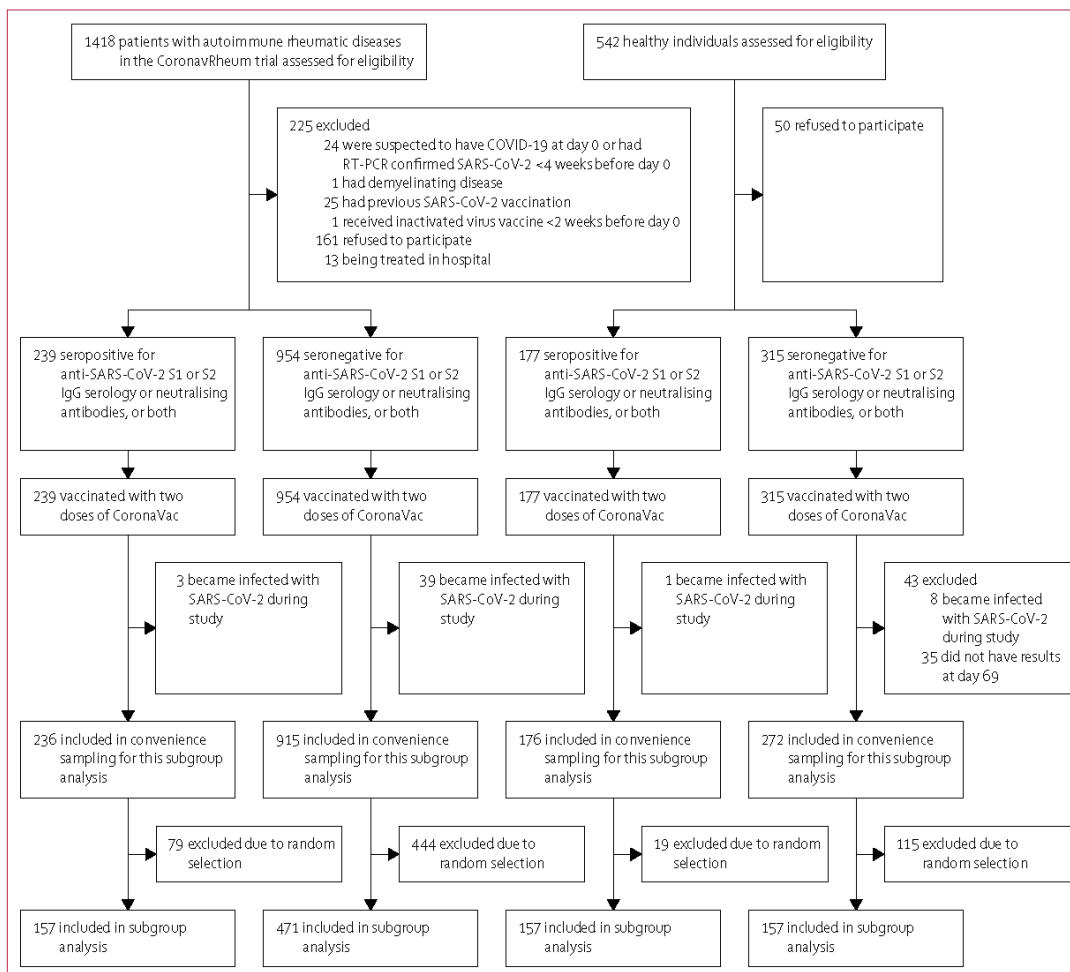


Figure 1: Study profile
S=spike.

	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					
Female	99 (63%)	297 (63%)	99 (63%)	99 (63%)	..
Male	58 (37%)	174 (37%)	58 (37%)	58 (37%)	..
Race					
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 immunosuppressive 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 $\ln(\text{IgG})$]) 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 5.7 AU/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

	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 $\geq 30\%$. 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

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 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).

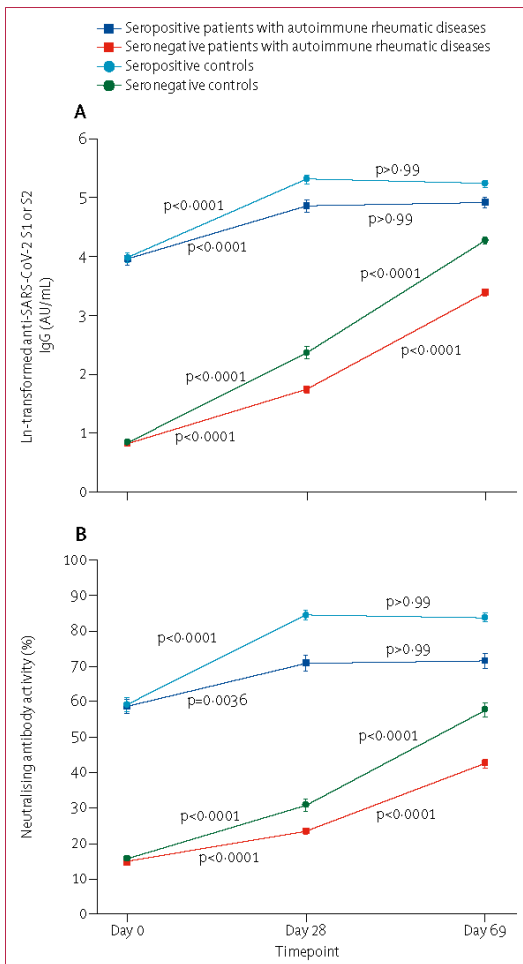


Figure 2: Anti-SARS-CoV-2 S1 or S2 IgG GMTs (A) and neutralising antibody activity (B) before (day 0) and after the first (day 28) and second (day 69) doses of CoronaVac. Datapoints are mean values, with error bars showing SD. The minimum possible value for anti-SARS-CoV-2 S1 or S2 IgG is 0.64 (ln 1.9, 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.

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

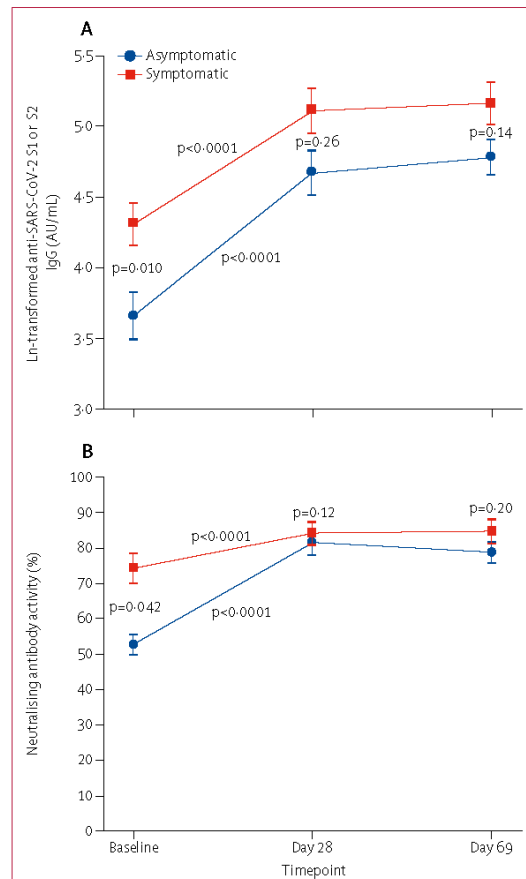


Figure 3: Anti-SARS-CoV-2 S1 or S2 IgG GMTs (A) and neutralising antibody activity (B) before (day 0) and after the first (day 28) and second (day 69) doses of CoronaVac in seropositive patients with autoimmune rheumatic diseases who had symptomatic infection (n=41) versus asymptomatic infection (n=73). 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.

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 infection¹⁴ 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 vaccine⁴ 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 colleagues⁹ 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.

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4.7. CoronaVac produces antibodies in 87% of the patients with Hepatitis B, demonstrates chinese study

CoronaVac, a vaccine from Butantan and the chinese pharmaceutical Sinovac, generates a high protection against Covid-19 on patients that live with Hepatitis B without causing severe adverse reactions. The conclusion is part of a study published by Chinese researchers in Cellular & Molecular Immunology journal, from Nature. According to the research, after receiving the second dose of the immunizer, the patients presented a seroconversion tax of 87,25% for IgG antibodies, and of 74,5% for neutralizing antibodies.

The article “Safety and immunogenicity of a SARS-CoV-2 inactivated vaccine in patients with chronic hepatitis B virus infection” was conducted by researchers from the Medicine School of the Huazhong University of Science and Technology, from Wuhan, China, where the Covid-19 pandemic started.

The study was made with 284 patients with chronic infection of hepatitis B, and 81 of them were not vaccinated, 54 had received just the first dose of the vaccine, and 149 of them had completed the vaccinal scheme of two doses. One month after the first or second dose,

plasma samples were collected and compared to the samples of the non vaccinated.

While the serum positivity in the vaccinated for the IgG antibodies and the neutralizing antibodies were 87,5% and 74,5%, respectively, the data of adverse reactions demonstrated that almost every reaction was mild, and the most common symptom was local pain in the area of injection followed by somnolence. Only one patient reported fever on the first day after the vaccination. There were no severe adverse reactions observed even on the 20 patients with more serious cases of chronic infection of hepatitis B (abnormal levels of alanine aminotransferase) or in the 10 patients with hepatic cirrhosis.

This is the first detailed study that analyzes the safety and immunogenicity of CoronaVac in patients with chronic infection of hepatitis B. Previous studies demonstrated a higher risk of progression for the severe disease on people with cirrhosis infected with the SARS-CoV-2 virus.

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CORRESPONDENCE OPEN



Safety and immunogenicity of a SARS-CoV-2 inactivated vaccine in patients with chronic hepatitis B virus infection

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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- λ 3 [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.

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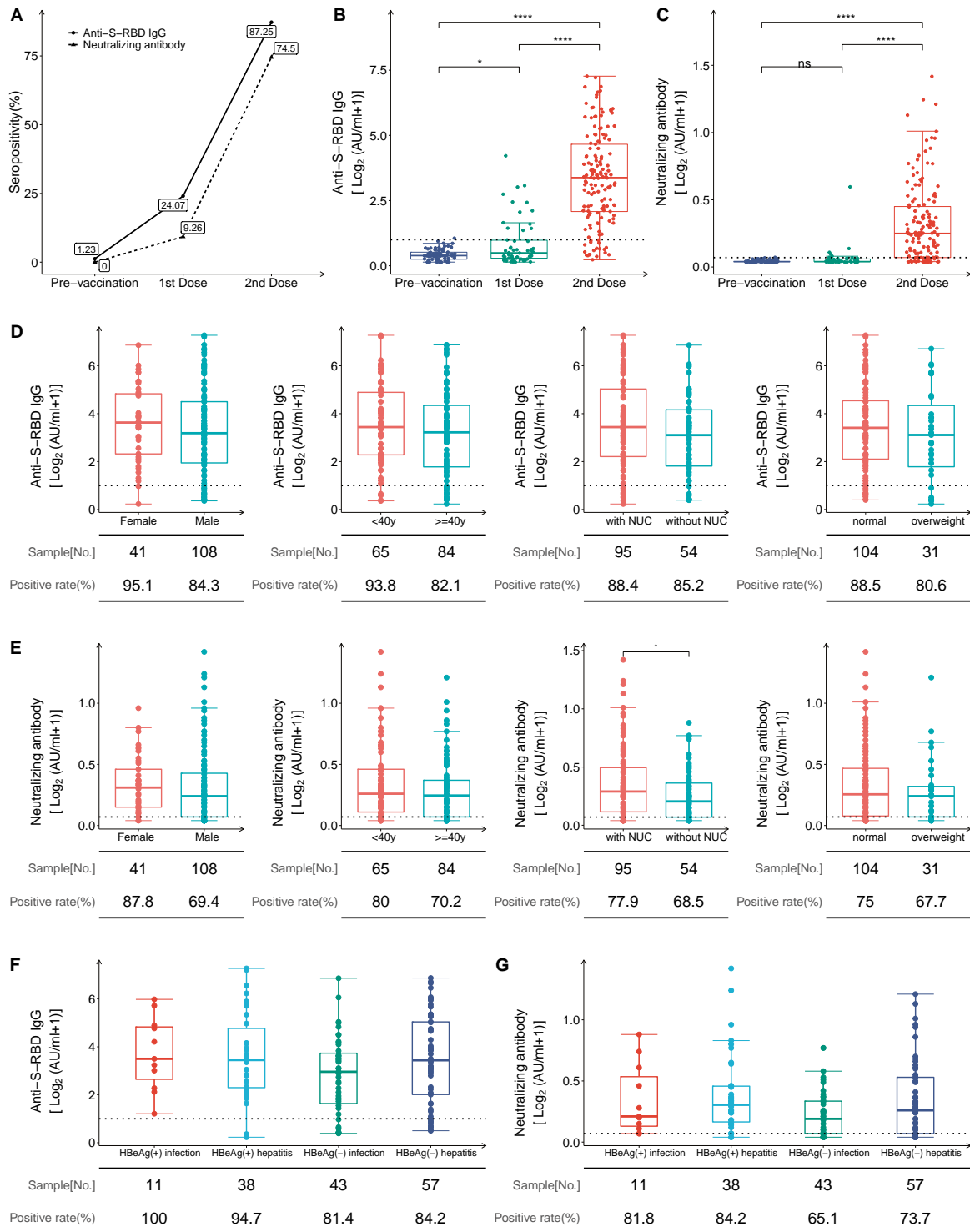


Fig. 1 Antibody responses following immunization with the inactivated vaccine in CHB patients. **A** The seropositivity of anti-S-RBD-IgG and NABs in CHB patients. **B, C** Kinetics of the anti-S-RBD-IgG and NAb titers in vaccine-induced sera at different time points in CHB patients. Prevaccination, $n = 81$; 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.

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AUTHOR CONTRIBUTIONS

XZ, BJW, TDX, and BYL designed and conceived the study; TDX, BYL, and HW performed the experiments; TDX, BYL, HW, XFX, 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>.

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4.8. CoronaVac is safe and immunogenic for patients with systemic autoimmune myopathies

A clinical study of phase 4 conducted by the Medicine School of the University of Sao Paulo, published in the scientific journal *Rheumatology*, presented evidence that CoronaVac is safe and induces immune response in patients with systemic autoimmune myopathies (SAM). This is a heterogeneous group of rare systemic diseases that mainly affect the skeletal striated muscles, and may also affect the lungs, heart and gastrointestinal tract.

Six weeks after completing the vaccinal scheme of two doses from CoronaVac, the 37 patients that participated in the research presented an average activity neutralization similar to 79 control individuals non immunocompromised (57,2% vs 63%). And the frequency of neutralizing antibodies production was 51,4% on the patients and 77,2% on the controls.

In comparison to the production of IgG antibodies, 64,9% of the

patients presented seroconversion, with an average geometric titration of IgG antibodies of 7,9.

The authors of the study emphasized that, besides presenting a smaller immunogenicity in comparison to healthy people, which is something expected of immunosuppressed individuals, the patients developed a good response to SARS-CoV-2. Besides, no severe adverse effects were observed, proving the safety of CoronaVac on that population. The frequency of mild adverse reactions was similar in both groups.

During the monitoring, six individuals (three patients and three controls) had Covid-19, five of them between the first and second dose and only one after the second dose. All of them developed mild symptoms and there was no need of hospitalization.

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Original article

Systemic autoimmune myopathies: a prospective phase 4 controlled trial of an inactivated virus vaccine against SARS-CoV-2

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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.

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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 ± 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 μ g 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. Secondly, 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.

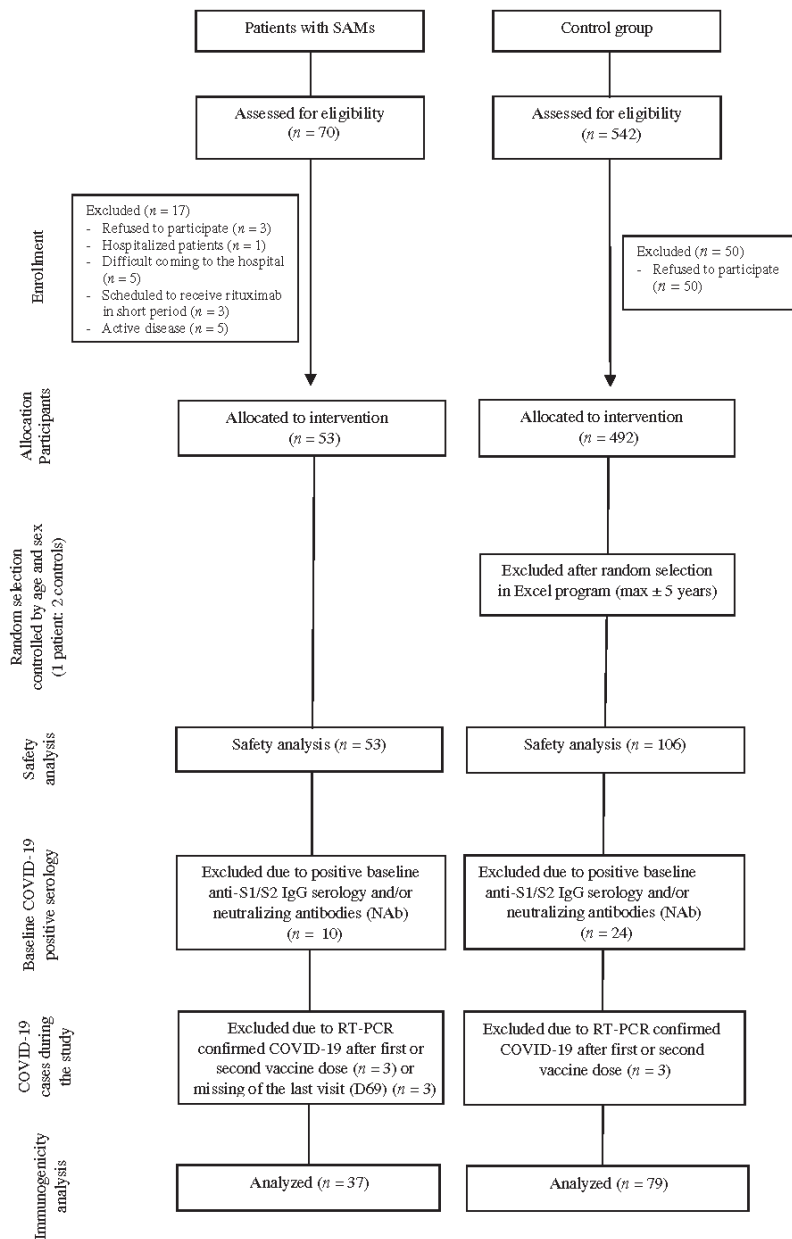
Anti-SARS-CoV-2 S1/S2 IgG antibodies

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 $\geq 30\%$) 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 ≥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. ^aLast 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		After vaccine			After vaccine	
	First dose		First dose (D28)		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. ^aP<0.001 for longitudinal comparison of GMT in SAMs at D69 vs baseline. ^bP<0.001 for longitudinal comparison of GMT in SAMs at D69 vs D28. ^cP<0.001 for longitudinal comparison of GMT in controls at D28 and D69 vs baseline. ^dP<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. ^aP<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 non-linear 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						
Mycophenolate mofetil	19 (79.2)	13 (100)	0.140	14 (73.7)	18 (100)	0.046
MTX	7 (29.2)	8 (61.5)	0.056	6 (31.5)	9 (50)	0.254
AZA	7 (29.2)	1 (7.7)	0.216	5 (26.3)	3 (16.7)	0.693
LEF	4 (16.7)	2 (15.4)	1.000	3 (15.7)	3 (16.7)	>0.999
Ciclosporin	3 (12.5)	0	0.538	2 (10.5)	1 (5.6)	>0.999
CYC	0	2 (15.4)	–	0	2 (11.1)	–
Rituximab	1 (4.2)	1 (7.7)	1.000	1 (5.3)	1 (5.6)	1.000
	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	CTRL	P-value	SAMs	CTRL	P-value
	(n = 53)	(n = 106)		(n = 50)	(n = 106)	
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	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. ^aAt 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.

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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.

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CoronaVac

What science proves

4.9. CoronaVac generates high levels of protection for people with HIV, indicates studies from Brazil and China

Two scientific studies published by researchers from Brazil and China demonstrates that CoronaVac, a vaccine from Butantan and the Chinese pharmaceutical Sinovac against Covid-19, is safe and capable of generating high levels of protection against SARS-CoV-2 on people infected with the HIV virus, the responsible of AIDS (Acquired immunodeficiency syndrome).

The paper “Safety and Immunogenicity of CoronaVac in People Living with HIV”, written by researchers of the Clinical Hospital from the University of Sao Paulo Medical School and published in the preprint platform SSRN, evaluated the safety and immunogenicity of CoronaVac on 215 people that live with HIV, in comparison with 296 without known immunosuppression. All the participants received two doses of CoronaVac with a gap of 28 days.

Four weeks after the second dose of the vaccine, the percentage of participants with positivity for SC and NAb neutralizing antibodies

was as high for the HIV group as for the control group. No severe adverse reaction was reported during the study, for people with HIV or non immunosuppressed participants.

However, the researchers found differences on the immunogenicity parameters among people with HIV. The T CD4 lymphocytes (CD4 cells) help to coordinate the immune response, stimulating other immune cells such as B lymphocytes (B cells) and T CD8 (CD8 cells) to fight against the infection. The HIV virus weakens the immunological system, destroying the CD4 cells. 69 days after the first dose of CoronaVac, the participants with T CD4 cells count lower than 500 cells/mm³ had immunogenicity lower against the SARS-CoV-2 in comparison with the members of the same group with count higher or equal to 500 cells/mm³.

From that analysis, the researchers concluded that people with HIV and count higher or equal to 500 T CD4 cells for mm³ had 2,26 times

more chances to present positivity in the activity of the neutralizing antibodies when compared to the count of T CD4 cells by mm^3 minor than 500. In relation to the control group participants, this indicator was 3,21 times higher.

“Our results show that CoronaVac has a robust immunogenicity on people with HIV after two doses of the vaccine, but the response of antibodies on that population are a little lower than on non immunosuppressed individuals”, said the authors. “Strategies must be developed to improve the immunogenicity induced by the vaccine among people living with HIV, especially on the subgroup of with a lower count of T CD4 cells. A possible procedure is to use a booster dose of the vaccine or even administer a higher antigen titers per dose”, they concluded.

Another study realized by chinese researchers and published in the SSRN platform also brought evidences that CoronaVac is safe

for people living with the HIV virus, and that people living on that group, when fully immunized in the scheme of two doses of the Butantan vaccine, may reach a higher levels of protection against the SARS-CoV-2, similar to the ones observed on negative-HIV individuals.

Covid and HIV

A report published in July 2021 by the Joint United Nations Programme on HIV/Aids (UNAIDS) analyzed more than 168 thousand of people hospitalized with Covid-19 all over the world and concluded that the incidence of the most severe form of the disease and the number of deaths intra-hospital were higher on people that live with HIV, independent of the age, gender and comorbidities. It is estimated that more than 38 million people live with HIV all over the world, being 1 million of them in Brazil.

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Safety and immunogenicity of CoronaVac in people living with HIV

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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/mm³) 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/mm³ compared to those with ≥500 cells/mm³. 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 $<500/\text{mm}^3$, those with CD4+ counts $\geq 500/\text{mm}^3$ 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 $<500/\text{mm}^3$ are at increased risk for a blunted antibody response following vaccination.

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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 $<500 \text{ cells}/\text{mm}^3$ had lower SARS-CoV-2 immunogenicity compared to PLWH with CD4+ T cell counts $\geq 500 \text{ cells}/\text{mm}^3$ 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.

Article

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 covariates⁴, 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 B⁶, 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/mm³.⁹ 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

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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 $\geq 30\%$ 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/mm³ for 64 (30%) participants and ≥ 500 cells/mm³ 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).

Article

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/mm³ had a lower immunogenicity compared to those with CD4+ T cell counts ≥ 500 cells/mm³ [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 /mm³ 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/mm³, CD4+ ≥ 500 cells/mm³ and HIV-uninfected participants; the median final NAb activity was 41.6 % (IQR 20.8 – 64.6) among PLWH with < 500 cells/mm³; 49.9 % (IQR 30.6 – 73.1) for PLWH with ≥ 500 cells/mm³; 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 /mm³, 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

Article

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 titer



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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, 50th, 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

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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/mm³ had lower SARS-CoV-2 immunogenicity compared to PLWH with CD4+ T cell counts ≥ 500 cells/mm³.

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 /mm³ had significantly higher odds of having a positive NAb compared to PLWH with CD4+ T cell count < 500 /mm³.

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/mm³) 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 immunogenicity²³ 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/mm³. 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

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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 $>350/\text{mm}^3$. 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 influenza²⁶ 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 PLWH 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 PLWH, 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.

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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.

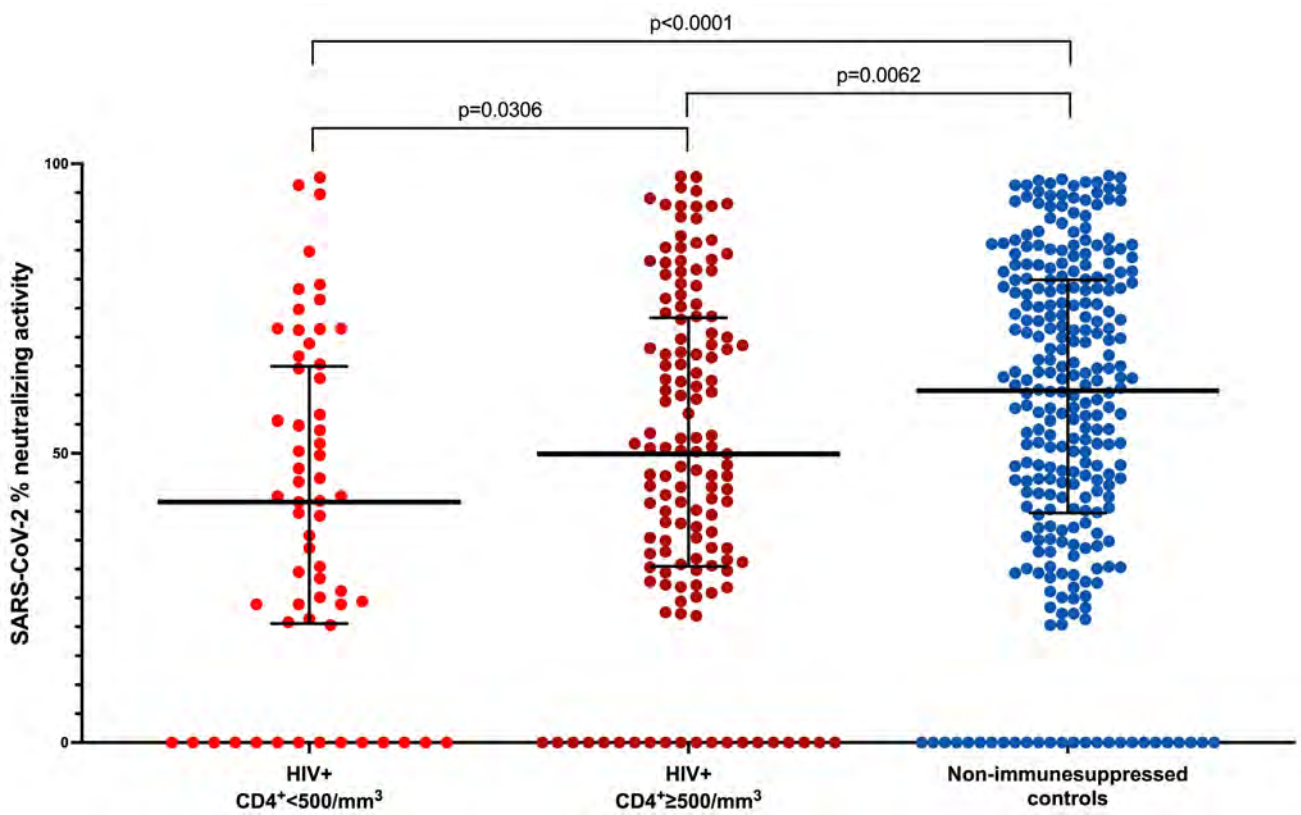
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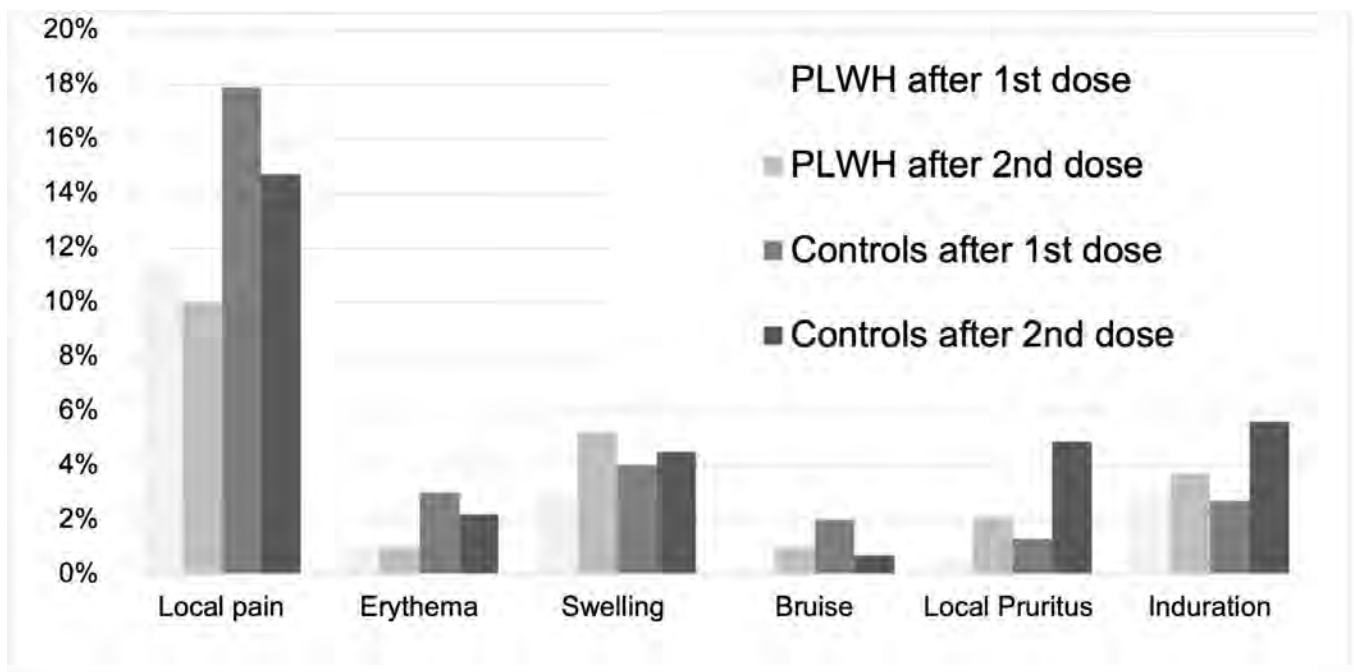
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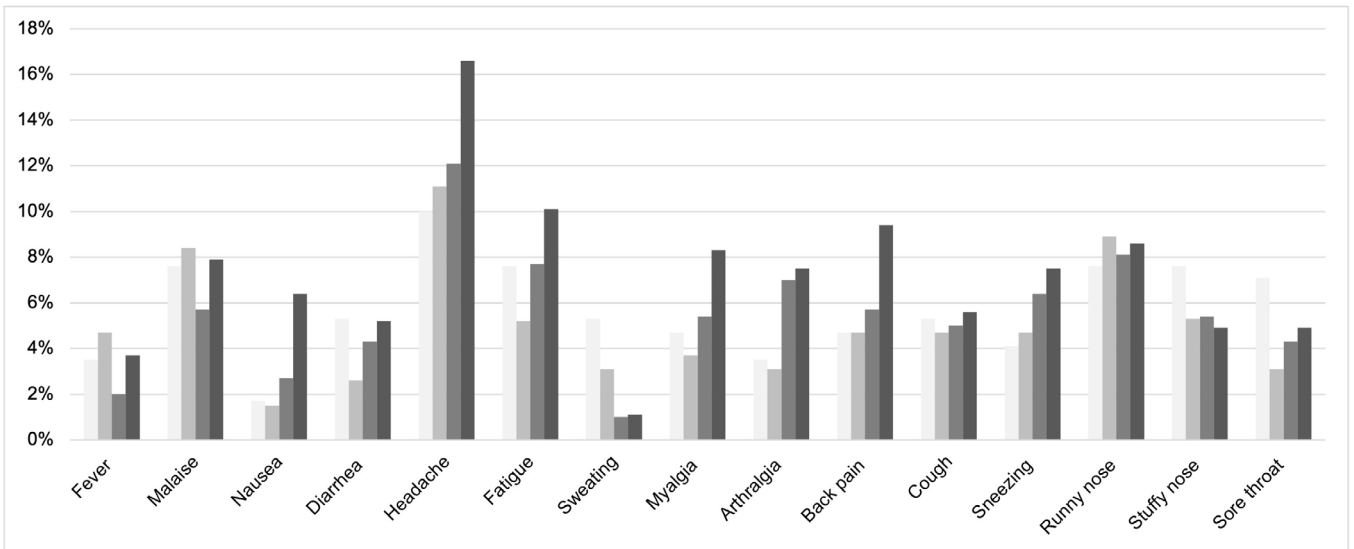
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1 **Comparing immune responses to inactivated vaccines against SARS-CoV-2 between**
 2 **people living with HIV and HIV-negative individuals: a cross-sectional study in China**

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46 **Running head:** Comparing immune responses to inactivated vaccines against SARS-CoV-2 between people
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52 **Summary**

53 **Background** There are concerns about the efficacy and safety of SARS-Cov-2 vaccines
54 among People living with HIV (PLWH). We compared immunogenicity and safety of the
55 inactivated SARS-CoV-2 vaccines (Sinopharm and Sinovac CoronaVac) between PLWH and
56 HIV-negative individuals.

57 **Methods** PLWH and HIV-negative individuals aged 18-59 years who had received at least
58 one dose of inactivated SARS-CoV-2 vaccine were recruited in two Chinese cities between
59 April and June 2021. Participants completed a self-administered questionnaire collecting
60 adverse events and background characteristics. Venous blood samples were collected and
61 tested for neutralizing antibody responses against authentic SARS-CoV-2, the total antibody
62 specific to SARS-CoV-2, SARS-CoV-2 IgG antibody against the receptor-binding domain of
63 the spike protein (S-IgG), and antigen-specific T-cell immune response level.

64 **Findings** A total of 129 PLWH and 53 HIV-negative individuals completed this study.
65 Prevalence ($P=0.19$) and severity ($P=0.13-0.77$) of adverse events were similar among
66 PLWH and HIV-negative individuals. The prevalence of seropositivity of neutralizing
67 antibody, total antibody and S-IgG was 71.3%, 81.9% and 92.5% among fully vaccinated
68 PLWH, which is similar to fully vaccinated HIV-negative individuals ($P=0.07-0.48$). Among
69 all participants, PLWH had significantly lower neutralizing antibody, total antibody, S-IgG,
70 and T-cell specific immune response levels compared to HIV-negative individuals, after
71 controlling for types of vaccine, time interval between prime and second dose, time after
72 receiving the second dose, and sociodemographics. PLWH who had a longer time since HIV
73 diagnosis, completed the second dose for 15-28 days, and an interval between prime and
74 second dose of ≥ 21 days had higher neutralizing antibody levels.

75 **Interpretation** Inactivated SARS-CoV-2 vaccines are safe for PLWH. Fully vaccinated
76 PLWH could achieve similarly high protection as HIV-negative individuals. Vaccination
77 guidelines for PLWH should be developed.

78 **Funding** Beijing Excellent Talent Plan, Beijing Talent Project in the New Millennium, the
79 National Institute of Mental Health of the National Institutes of Health under Award.

80

81 **Keywords:** People living with HIV; Inactivated SARS-CoV-2 vaccines; self-reported
82 adverse events; neutralizing antibody responses against authentic SARS-CoV-2; total
83 antibody specific to SARS-CoV-2; SARS-CoV-2 IgG antibody; antigen-specific T-cell
84 immune response.

85 **Introduction**

86 Globally, about 38 million people are living with HIV ¹. Antiretroviral therapy (ART) could
87 suppress viral replication, restore CD4⁺ T-cell counts, rebuild immune function, and decrease
88 morbidity and mortality among people living with HIV (PLWH) ^{2,3}. However, CD4⁺ T-cell
89 recovery is incomplete despite viral suppression in some PLWH ⁴. The World Health
90 Organization (WHO) confirmed that HIV infection is a significant independent risk factor for
91 both severe SARS-CoV-2 cases at hospital admission and in-hospital mortality ⁵. Both
92 international health authorities and Chinese national guidelines recommend SARS-CoV-2
93 vaccination to PLWH regardless of their immune status ⁶⁻⁸.

94 PLWH is considered a priority group for vaccination in many countries ⁸. However, there are
95 concerns that PLWH might have a suboptimal response to SARS-CoV-2 vaccination. More
96 importantly, less than 3% of the participants in the reported SARS-CoV-2 vaccine efficacy
97 trials are PLWH, and the data for vaccine safety and immune response is insufficient ⁹⁻¹³. The
98 Novavax study showed the overall vaccine efficacy was higher when excluding PLWH from
99 the analysis (increased from 49.4% to 60%) ¹³. Most studies did not report vaccine efficacy
100 specific for PLWH. Some studies have compared the safety and immunogenicity of mRNA
101 (Pfizer BNT162b2 and Moderna mRNA-1273) or adenovirus vector (Oxford/AstraZeneca
102 AZD1222) SARS-CoV-2 vaccines between HIV-negative individuals and PLWH with viral
103 suppression and high CD4⁺ T-cell levels (median around 700) ¹⁴⁻¹⁸. These studies showed that
104 SARS-CoV-2 vaccines were safe for PLWH, and there was no between-group difference in
105 adverse events ¹⁴⁻¹⁸.

106 There are two inactivated SARS-CoV-2 vaccines manufactured by Chinese companies are
107 approved for emergency use by the WHO (Sinopharm and Sinovac CoronaVac) ^{19,20}. More
108 than three billion doses of these vaccines has been supplied to more than 40 countries ²¹. No
109 study compared PLWH and HIV-negative individuals regarding immunogenicity and safety
110 of the inactivated SARS-CoV-2 vaccines. Such evidence is important to address COVID-19
111 vaccine hesitancy among PLWH or to implement boost dose for this group ²². Previous
112 findings on mRNA/adenovirus vector vaccines might not be applicable to PLWH receiving
113 inactivated SARS-CoV-2 vaccines ¹⁴⁻¹⁸. Moreover, it is unclear whether PLWH with lower
114 CD4⁺ T cell counts and detectable HIV viral load would have similar immunogenicity as
115 HIV-negative individuals, as these PLWH were excluded by the aforementioned studies ¹⁴⁻¹⁸.
116 Furthermore, given the relatively short follow-up period in previous studies, there is no

117 consensus about the long-term immunogenicity to SARS-CoV-2 vaccines among PLWH¹⁴⁻
118 ¹⁸.

119 This study aims to address these knowledge gaps by comparing the immunogenicity and
120 adverse events between PLWH and HIV-negative individuals after vaccination. This study
121 also investigated factors correlated with levels of neutralizing antibody responses against
122 authentic SARS-CoV-2, the total antibody specific to SARS-CoV-2, SARS-CoV-2 IgG
123 antibody against the receptor-binding domain (RBD) of the spike protein (S-IgG), and
124 antigen-specific T-cell immune response among PLWH.

125

126 **Methods**

127 **Study design**

128 This cross-sectional study was conducted in two Chinese metropolitan cities (Beijing and
129 Tianjin) conducted between April and June 2021. Participants included PLWH and HIV-
130 negative individuals who have received at least one dose of inactivated SARS-Cov-2 vaccine.

131 **Participants**

132 The inclusion criteria for PLWH included: 1) aged 18-59 years, 2) willing to participate in the
133 study activities, including survey and blood sample collection, and relevant laboratory
134 testing, 3) having received at least one dose of inactivated SARS-CoV-2 vaccine (Sinovac
135 CoronaVac or Sinopharm), and 4) having received HIV diagnosis confirmed by HIV-1/2
136 western blot assay. Exclusion criteria included: 1) presence of severe hearing loss, impaired
137 vision, or intellectual disability observed by the interviewers, and 2) history of SARS-CoV-2
138 infection, major psychiatric illness (schizophrenia and bipolar disorder) or neurocognitive
139 impairment based on clinician's assessment of their medical records. HIV-negative
140 individuals shared the first three inclusion criteria and both exclusion criteria with PLWH.
141 HIV serostatus was confirmed by Abbott ARCHITECT HIV Ag/Ab Combo assay.

142 **Recruitment and data collection**

143 Recruitment for PLWH was facilitated by two community-based organizations (CBOs), one
144 in each city. These two CBOs have provided services to PLWH and HIV high-risk
145 populations and worked closely with HIV clinical service providers. WeChat is the most
146 commonly used social media application for the CBOs to communicate with PLWH clients.
147 CBO staff posted the study recruitment information in the WeChat public accounts of their
148 organizations. Interested PLWH contacted CBO staff through private WeChat messages,
149 phone calls, and messages via other instant messaging applications. CBO staff screened

150 participants' eligibility, briefed them about the study purpose and procedures, assured them
151 that identifiable information would be kept confidential, and refusal to participate would have
152 no consequences. The recruitment of HIV-negative individuals was conducted in community
153 hospitals. The hospital staff approached vaccinated individuals in their service records by
154 telephone and invited them to participate.

155 PLWH and HIV-negative individuals interested in joining the study were invited to visit one
156 of two clinics, one in each city. On-site, project staff obtained their written informed consent.
157 All participants completed a 10-minute self-administered questionnaire on site. The STROBE
158 checklist was adhered (see Appendix).

159 **Blood sample collection and laboratory procedures**

160 After completion of the survey, trained nurses collected two lithium heparin anticoagulated
161 vacuum blood collection tubes (BD) of whole blood (10 ml), two EDTA anticoagulated
162 vacuum blood collection tubes (BD) of whole blood (10 ml), and one SST blood collection
163 tube of whole blood (5ml). One tube of lithium heparin salt anticoagulated whole blood and
164 one tube of EDTA anticoagulated whole blood were placed at room temperature. They were
165 assayed for T cell-specific immune response within 8 hours and CD4⁺ T-cell count within 48
166 hours, respectively. The other three tubes of whole blood were centrifuged at 1300 relative
167 centrifugal force (RCF) for 10 minutes, and the upper plasma/serum layers were transferred
168 into lyophilized tubes of no less than 1.2 ml each, and were stored at -20°C for the detection
169 of SARS-Cov-2 combined antibody and neutralizing antibody, as well as HIV viral load.

170 SARS-CoV-2 neutralizing antibody measurement. The neutralizing antibodies to authentic
171 SARS-CoV-2 (virus strain SARS-CoV-2/human/CHN/CN1/2020, GenBank number
172 MT407649.1) were quantified using a micro cytopathogenic effect (CPE) inhibition assay
173 with a minimum four-fold dilution as reported before²³. The positive geometric mean titer
174 (GMT) of the neutralizing antibodies to authentic SARS-CoV-2 was 8.

175 SARS-CoV-2 antigen/antibody combined testing. All samples were tested for total antibody
176 and SARS-CoV-2 specific S-IgG antibodies using Chemiluminescence assay (CLIA) kits
177 (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.). The positive cut-off for the
178 abovementioned tests was 1.0.

179 T-cell specific immune response. The T cell specific immune response was tested using the
180 IFN- γ release assay (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.). Briefly, 1.5
181 ml of heparin blood was distributed into test tube containing specific SARS-CoV-2 S antigen
182 (T tube), negative control tube (N tube), and positive control tube (P tube) within 8 hours.

183 The tubes were inverted and mixed 5 times, incubated in 37°C for 20-24 hours. Then the
184 plasma was collected after centrifuging at 3000 RCF for 10 minutes and detected for IFN- γ
185 level. Level of T tube minus N tube, a value greater than 30 pg/ml was considered positive.
186 HIV viral load detection Viral load of PLWH was tested using HIV quantitative assay
187 (Zhuhai Livzon Diagnostics Inc.). The limit of quantitation (LOQ) of this assay was 60
188 copies/ml.

189 CD4⁺ cell count measurement. The assay was performed using flow cytometry testing
190 methods (BD Biosciences, San Jose, CA, USA) in accordance with the China National
191 Guideline for Detection of HIV/AIDS (version 2020) ²⁴.

192 Background characteristics of the participants. All participants reported age, gender, and
193 presence of chronic conditions. Characteristics related to HIV infection and SARS-CoV-2
194 vaccination were extracted from medical records.

195 Adverse events related to SARS-CoV-2 vaccination. A checklist was used to assess local
196 adverse events (pain, redness, itch, swelling, induration, and skin rash in the arm where the
197 shot was given) and systematic adverse events (fatigue, malaise, headache, dizziness,
198 lethargy, joint pain or muscle ache, feverish, nausea, vomit, diarrhea, and others) within one
199 month after receiving SARS-CoV-2 vaccines. Participants rated the severity the
200 aforementioned adverse events (1=very mild, 2=mild, 3=moderate, 4=severe, and 5=very
201 severe).

202 **Sample size planning**

203 Previous studies showed that the positive rate for SARS-CoV-2 neutralizing antibody was
204 about 90% among HIV-negative individuals who received inactivated SARS-CoV-2 vaccines
205 ²³. There was no data on seropositivity for SARS-CoV-2 neutralizing antibody among PLWH
206 who received inactivated vaccines. Previous studies showed that the seroconversion rate of
207 PLWH after inoculation of the hepatitis B vaccine ranged from 34% to 88% ²⁵. Therefore, we
208 assumed 70% of vaccinated PLWH would be positive for SARS-CoV-2 neutralizing
209 antibody. Using an allocation ratio of 2:1, a total of 102 PLWH and 51 HIV-negative
210 individuals was required to detect a minimum between-group difference of 20% (90% versus
211 70%) in SARS-CoV-2 neutralizing antibody positive rate ($\alpha=0.05$, $\beta=0.10$).

212 **Statistical analysis**

213 Chi-square tests were used to inspect the difference in background characteristics and adverse
214 events related to SARS-CoV-2 vaccination between PLWH and HIV-negative individuals.
215 Between-group differences in immunogenicity indicator levels (total antibody, neutralizing

216 antibody, S-IgG, and T-cell specific immune response) were tested using Mann-Whitney
217 tests. We log transformed the immunogenicity indicator levels using the base of 10 to
218 normalize the data. Multivariable linear regression models were performed to test the
219 between-group difference in these indicators, after controlling for all background
220 characteristics with $P < 0.05$ in between-group comparisons. Adjusted coefficients (B) were
221 obtained. Moreover, same comparisons were performed between different subgroups of
222 PLWH and HIV-negative individuals. Similar analyses on sero-positivity for these
223 immunogenicity indicators was also performed. Among PLWH, linear regression models
224 were used to inspect factors that were correlated with immunogenicity indicator levels. SPSS
225 version 26.0 was used in all analyses, with two-tailed $P < 0.05$ was considered statistically
226 significant.

227 **Ethics approval**

228 Written informed consent was obtained from all participants before their study participation
229 in accordance with the Declaration of Helsinki. The Institutional Review Boards of Changzhi
230 Medical College (RT2021002) and Beijing Youan Hospital Research Ethics Committee (No.
231 2021-031) approved this study.

232 **Role of funding sources**

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239 Funders had no role in the design, data collection, analysis, interpretation of the study, or the
240 preparation of the manuscript.

241

242 **Results**

243 **Profiles of the participants**

244 A total of 519 and 316 PLWH in Beijing and Tianjin were approached, 130 and 24 were
245 screened to be eligible, and 110 (84.6%) and 19 (79%) completed the study. At the same
246 period, 61 vaccinated HIV-negative individuals were approached, 8 (13.1%) refused to
247 participate mainly due to logistic reasons, and 53 (86.9%) completed the study procedures.

248 Most PLWH received HIV diagnoses for more than one year (86%), and were on ART
249 (97.7%). Over half of them had an undetectable viral load (58.1%), and the median CD4⁺ T-
250 cell count was 630.5 (IQR: 499.5, 848.8) (Table 1).

251 As compared to HIV-negative individuals, fewer PLWH were 50-59 years old (3.9% versus
252 17.0%, $P=0.01$) and female (0.8% versus 24.5%, $P<0.001$). More PLWH had chronic
253 conditions (20.9% versus 0%, $P<0.001$), received Sinovac-CoronaVac (55.0% versus
254 30.2%, $P<0.001$) and only completed the prime dose (27.1% versus 3.8%, $P<0.001$).

255 Receiving more than one type of vaccine was not observed. Among those who completed
256 both doses, the time interval between the prime and second dose was shorter among PLWH
257 than HIV-negative individuals were (median: 21 versus 27 days, $P<0.001$) (Table 1). These
258 background characteristics were controlled when comparing immunogenicity indicators
259 levels between PLWH and HIV-negative individuals.

260 **SARS-CoV-2 vaccination adverse events**

261 Among the participants, 45.0% of PLWH and 54.7% of HIV-negative individuals reported
262 presence of any specific local and systematic adverse events. After controlling for significant
263 background characteristics (i.e., age group, gender, presence of chronic conditions other than
264 HIV, types of vaccine, time interval between prime and second dose, and time after receiving
265 the second dose), there is no between-group difference in prevalence of any adverse events
266 (AOR: 0.77, 95%CI: 0.31, 1.95, $P=0.19$). Most of the reported adverse events were very
267 mild/mild (41-100% among PLWH and 62.2-100% among HIV-negative individuals). There
268 was no between-group difference in the severity of these adverse events ($P=0.13-0.77$).
269 (Table 2)

270 Subgroup analysis showed that PLWH did not have a higher prevalence of any adverse
271 events when comparing with HIV-negative individuals, regardless of CD4⁺ T-cell counts or
272 HIV viral suppression status (Appendix 1).

273 **Immunogenicity indicators level**

274 The prevalence of seropositivity of neutralizing antibody, total antibody and S-IgG was
275 71.3%, 81.9% and 92.5% among fully vaccinated PLWH. Such prevalence is similar to that
276 observed among fully vaccinated HIV-negative individuals ($P=0.07-0.48$). (Appendix 2).

277 When comparing to HIV-negative individuals, PLWH had significantly lower levels of
278 neutralizing antibody (adjusted B: -0.18, $P=0.049$), total antibody (adjusted B: -0.80,
279 $P<0.001$), S-IgG (adjusted B: -0.31, $P=0.002$), and T-cell specific immune response
280 (adjusted B: -0.64, $P=0.002$). Subgroup analyses showed that PLWH with detectable viral
281 load (adjusted B: -0.29, $P=0.047$) or CD4⁺ T cell counts <500 (adjusted B: -0.29, $P=0.02$)

282 had significantly lower neutralizing antibody levels. Such difference in neutralizing antibody
283 level was not observed when comparing HIV-negative individuals with PLWH with
284 undetectable viral load or CD4⁺ T cell counts ≥ 500 . In addition, PLWH had significantly
285 lower levels of total antibody, S-IgG, and T-cell specific immune response regardless of
286 CD4⁺ T cell counts or HIV viral suppression. Neutralizing antibody levels among fully
287 vaccinated PLWH did not lower than fully vaccinated HIV-negative individuals (adjusted B:
288 -0.15 , $P=0.13$) (Table 3 & 4).

289 **Factors associated with immunogenicity indicator levels among PLWH**

290 A longer time since HIV diagnosis was associated with higher neutralizing antibody and total
291 antibody levels (2-5 years: adjusted B: 0.71 & 0.27 ; reference: ≤ 1 year). As compared to
292 partially vaccinated participants, PLWH who completed the second dose for 15-28 days had
293 higher neutralizing antibody levels (adjusted B: 0.30), while those who completed it for 15-
294 56 days had higher total antibody (adjusted B: 1.00), S-IgG (adjusted B: 0.53), and T-cell
295 specific immune response levels (adjusted B: $0.89-0.99$). Compared to PLWH with a time
296 interval of <21 days between the prime and second dose, those with an interval of 21-28 days
297 and >28 days had higher neutralizing antibody (adjusted B: 0.37 & 0.36), total antibody
298 (adjusted B: 1.22 & 1.28), and S-IgG levels (adjusted B: 0.43 & 0.53) (Table 5).

299

300 **Discussion**

301 Understanding the differences of immunoresponse between HIV negative and positive
302 individuals is essential in planning the SARS-CoV-2 vaccination for PLWH. We found the
303 levels of adverse events are comparable between PLWH and HIV-negative individuals. The
304 prevalence of seropositivity of neutralizing antibody, the total antibody, and S-IgG were
305 similarly high among fully vaccinated PLWH and HIV-negative individuals. However,
306 PLWH had lower immunogenicity indicator levels than HIV-negative individuals after
307 controlling for types of vaccine, time since receiving the prime dose, time interval between
308 prime and second dose, and socio-demographics. Our findings filled the knowledge gap on
309 the immune responses to SARS-CoV-2 vaccines among PLWH. It contributed critical
310 evidence to policymaking and vaccination program planning for countries that mainly using
311 inactivated SARS-CoV-2 vaccines.

312 Similar to studies on mRNA/adenovirus vector SARS-CoV-2 vaccines¹⁴⁻¹⁸, there was no
313 between-group difference in prevalence ($P=0.19$) or severity ($P=0.13-0.77$) of self-reported

314 adverse events. Most of the reported adverse events were very mild/mild among PLWH (41-
315 100%). Therefore, inactivated SARS-CoV-2 vaccines are safe for PLWH.

316 Four immunogenicity indicator levels were significantly lower among PLWH at 0-14 days
317 after receiving the second dose. PLWH might take longer to develop humoral and cellular
318 immune responses to inactivated SARS-CoV-2 vaccines. Previous case reports observed a
319 prolonged course of antibody development among PLWH infected with SARS-CoV-2 ²⁶.
320 Similar to HIV-negative individuals and PLWH who received other SARS-CoV-2 vaccines,
321 the studied immunogenicity indicators peaked at 15-56 days after the second dose among
322 PLWH ¹⁴⁻¹⁸. However, the peak levels of these indicators were lower among PLWH,
323 especially for total antibody and S-IgG. A faster decline in immune responses were also
324 observed among PLWH. All four immunogenicity indicators levels declined >56 days after
325 receiving the second dose among PLWH, while these indicators remained stable among HIV-
326 negative individuals even 84 days after the second dose. This study observed significantly
327 lower total antibody and S-IgG levels among PLWH >56 days after the second dose. B-cell
328 dysfunction caused by HIV gp120 binds directly to primary B-cell, and impaired cellular
329 immunity caused by CD4⁺ T cell depletion among PLWH might explain slower development,
330 lower peak levels, and faster decline of both humoral and cellular immune responses to
331 SARS-CoV-2 vaccines ^{27,28}. Such findings indicated that PLWH might need a boost dose
332 after the initial doses, and might need it earlier than HIV-negative individuals do. Future
333 studies with large sample size are needed to investigate long-term changes in these
334 immunogenicity indicators among PLWH.

335 Neutralizing antibody plays an important role in SARS-CoV-2 clearance and is a key
336 indicator for protection after vaccination ²⁹. We found that the seropositivity and levels of
337 neutralizing antibody was similarly high among fully vaccinated PLWH and HIV-negative
338 individuals. It implied that both groups obtained good protection against SARS-Cov-2 after
339 the vaccination and PLWH should complete both doses of vaccination as required. Subgroup
340 analysis showed that in line with studies using mRNA and/or adenovirus vector SARS-CoV-
341 2 vaccines, PLWH with higher CD4⁺ T-cell counts or undetectable viral load did not had
342 significantly lower neutralizing antibody level than HIV-negative individuals ¹⁴⁻¹⁸. However,
343 PLWH with lower CD4⁺ T-cell counts (<500) or detectable viral load had lower neutralizing
344 antibody level. Such findings added knowledge to immune responses to SARS-CoV-2
345 vaccines among PLWH with severer immunodeficiency. PLWH with severer
346 immunodeficiency should be encouraged to receive SARS-CoV-2 vaccines. In contrast to
347 findings on other types of vaccines, our study observed significant lower total antibody, S-

348 IgG, and T-cell specific immune responses levels among PLWH compared to HIV-negative
349 individuals. The difference could not be fully explained by the larger proportion of PLWH
350 with low CD4⁺ T-cell counts or detectable HIV viral load in this study. These indicators
351 were lower among PLWH regardless of their CD4⁺ T-cell counts or HIV viral load. Future
352 studies should compare PLWH's immunogenicity to different types of SARS-CoV-2
353 vaccines in order to determine the optimal choice for PLWH.

354 Compared to newly diagnosed PLWH, those who had been diagnosed for 2-5 years had
355 higher neutralizing antibody and total antibody levels. It is possible that these PLWH had
356 better functioning immune system after years of ART. It also highlighted the needs to further
357 increase HIV testing coverage among key population to early identify HIV infection and link
358 them to treatment and care. It will hence improve the effectiveness of SARS-CoV-2
359 vaccination for PLWH. Moreover, our results also suggested that, PLWH had a longer
360 interval between the prime and second dose (21-28 days or >28 days) had significantly higher
361 neutralizing antibody, total antibody and S-IgG levels compared to those with a shorter
362 interval. Existing guidelines of SARS-CoV-2 vaccination for PLWH did not mention the
363 optimal vaccination interval. Our findings suggested that future SARS-CoV-2 vaccination
364 program for PLWH should consider a longer interval between doses. More research is needed
365 to determine an optimal interval between doses for PLWH.

366 The study has several strengths. First, all participants underwent humoral and cellular
367 immune responses analysis in this study. Second, this study included a diverse sample of
368 PLWH with different CD4⁺ T cell level and HIV viral load. It filled the knowledge gaps
369 about immunogenicity to SARS-CoV-2 vaccines among PLWH with impaired functional
370 immune system and poorer control of HIV. Third, impact of between-group difference in
371 background characteristics on immunogenicity might be limited in this study, as background
372 characteristics were controlled during the comparison. Furthermore, this is also one the first
373 studies that assessed relationships between characteristics of PLWH and immunogenicity to
374 SARS-CoV-2 vaccines.

375 This study also has some limitations. First, this was a cross-sectional study. Possible changes
376 in immunogenicity indicator levels over time were unclear. Such study design cannot
377 establish causal relationship as well. Second, we did not use matching to sample HIV-
378 negative individuals according to PLWH's characteristics. There are significant between-
379 group differences in socio-demographics, presence of other chronic conditions, and
380 vaccination characteristics. We controlled these characteristics when comparing the between-
381 group difference in immunogenicity. Third, PLWH was over-represented by male. However,

382 the impact of gender difference on immunogenicity might be limited, as previous studies did
383 not show difference in immunogenicity between male and female ²³. Moreover, the presence
384 and severity of adverse events were self-reported by participants and might be subject to
385 recall bias. We were not able to compare the safety data with other studies that used clinician
386 assessments.

387 Inactivated SARS-CoV-2 vaccines are safe for PLWH. Fully vaccinated PLWH could
388 achieve similarly high protection as HIV-negative individuals. PLWH had significantly lower
389 neutralizing antibody, total antibody, S-IgG, and T-cell specific immune response levels than
390 HIV-negative individuals did. The immunogenicity indicator levels peaked 15-56 days after
391 PLWH receiving the second dose. A longer time since diagnosis and a longer interval
392 between the prime and second dose were correlated with better immune responses among
393 PLWH. Future studies should compare PLWH's immunogenicity to different types of
394 vaccines, assess immune responses in a longer term, and investigate the optimal interval
395 between doses.

396

397 **Contributors**

398 All authors contributed to the conception of this study. XJH, WMT, and JJX developed the
399 methodology. DX, YY, XJ, JYD, MHY, LNW, and JJX were responsible for site survey and
400 coordination. XJH, BS, TZ, YY, LNW, and JJX were responsible for the laboratory testing
401 and test result interpretation. WMT, ZXW, XJZ, SMZ, YF, and JJX wrote the original draft.
402 All authors contributing to the reviewing and editing process. All authors agreed to submit
403 the manuscript for publication.

404 **Declaration of interests**

405 We declare no competing interests.

406 **Data sharing**

407 The individual participant data used in this analysis are available upon request. Requests
408 should be directed to the corresponding author, and need to sign a data access and
409 confidentiality agreement.

410 **Acknowledgments**

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412 PLWH who contribute to this research in the mainland of China. We are also grateful to local
413 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)	<i>P</i> 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 (1 st) 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

416 N.A.: not applicable.

417

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		P	Total antibody		P	S-IgG		P	T cell specific immune response		P
	PLWH	HIV-negative		PLWH	HIV-negative		PLWH	HIV-negative		PLWH	HIV-negative	
	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 (-0.57, 0.45)		0.06 (-0.14, 0.25)		0.03 (-0.26, 0.32)		-0.07 (-0.51, 0.38)	
40-49	0.17 (-0.52, 0.86)		0.08 (-0.18, 0.35)		-0.09 (-0.48, 0.31)		-0.08 (-0.69, 0.53)	
50-59	-0.32 (-1.51, 0.87)	---	-0.03 (-0.49, 0.43)	---	0.03 (-0.66, 0.71)	---	-0.56 (-1.62, 0.49)	---
Gender								
Male	Ref		Ref		Ref		Ref	
Female	1.47 (-1.02, 3.97)	---	0.77 (-0.18, 1.73)	---	0.63 (-0.81, 2.06)	---	1.14 (-1.07, 3.34)	---
Presence of chronic conditions other than HIV/AIDS								
No	Ref		Ref		Ref		Ref	
Yes	0.12 (-0.42, 0.66)	---	-0.07 (-0.28, 0.14)	---	-0.01 (-0.30, 0.32)	---	-0.20 (-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 (-0.12, 1.22)	0.71 (0.23, 1.19)**	0.21 (-0.05, 0.46)	0.27 (0.05, 0.48)*	0.21 (-0.18, 0.60)		0.46 (-0.14, 1.06)	
6-10	0.82 (0.10, 1.53)*	0.49 (-0.03, 1.00)†	0.33 (0.05, 0.60)*	0.23 (-0.01, 0.46)†	0.33 (-0.09, 0.74)		0.56 (-0.08, 1.19)†	
>10	0.59 (-0.20, 1.38)	0.44 (-0.13, 1.05)	0.22 (-0.08, 0.53)	0.15 (-0.11, 0.20)	0.23 (-0.23, 0.69)	---	0.55 (-0.15, 1.26)	---
Viral load (cp/ml)								
Undetectable								
61-200	Ref	Ref	Ref		Ref	Ref	Ref	Ref
>200	-0.39 (-0.89, 0.10)	-0.24 (-0.61, 0.14)	-0.17 (-0.36, 0.03)†		-0.33 (-0.61, -0.05)*	-0.19 (-0.40, 0.03)†	-0.15 (-0.61, 0.30)	-0.01 (-0.44, 0.42)
CD4+ T cell count (cells/μL)	-1.10 (-1.69, -0.51)***	-0.24 (-0.69, 0.22)	-0.23 (-0.47, 0.001)†	---	-0.68 (-1.01, -0.34)***	-0.24 (-0.50, 0.03)†	-0.60 (-1.14, -0.06)*	-0.27 (-0.78, 0.25)
<500	Ref		Ref		Ref		Ref	
500-1,000	0.41 (-0.10, 0.93)		0.13 (-0.07, 0.33)		0.16 (-0.14, 0.45)		0.59 (0.14, 1.04)*	0.47 (0.05, 0.89)*
>1,000	0.61 (-0.15, 1.36)	---	0.14 (-0.15, 0.44)	---	0.24 (-0.20, 0.68)	---	0.48 (-0.18, 1.14)	0.40 (-0.22, 1.01)
On ART								
No	Ref		Ref		Ref		Ref	
Yes	0.47 (-0.99, 1.93)	---	0.12 (-0.44, 0.68)	---	0.13 (-0.71, 0.97)	---	0.34 (-0.95, 1.62)	---
SARS-CoV-2 vaccination								

SARS-CoV-2 vaccination status	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Partially vaccinated	0-68	N.A.	0-27	N.A.	0-49	N.A.	0-07	0-16
0-14 days after fully vaccinated	(0-11, 1-25)*		(0-02, 0-51)*		(0-17, 0-80)**		(-0-55, 0-69)	(-0-46, 0-78)
15-28 days after fully vaccinated	2-11	1-00	0-72	0-30	1-26	0-53	1-08	0-99
	(1-68, 2-55)***	(0-43, 1-57)**	(0-54, 0-91)***	(0-04, 0-56)*	(1-02, 1-50)***	(0-20, 0-85)**	(0-61, 1-54)***	(0-50, 1-47)***
29-56 days after fully vaccinated	1-79	1-00	0-49	0-16	1-08	0-53	0-94	0-89
	(1-32, 2-27)***	(0-43, 1-57)**	(0-28, 0-69)***	(-0-10, 0-41)	(0-82, 1-35)***	(0-20, 0-85)**	(0-42, 1-45)***	(0-37, 1-40)**
57-84 days after fully vaccinated	1-36	0-85	0-38	0-14	0-75	0-26	-0-07	-0-10
	(0-74, 1-97)***	(0-15, 1-55)*	(0-12, 0-65)**	(-0-17, 0-45)	(0-40, 1-09)***	(-0-13, 0-65)	(-0-74, 0-59)	(-0-76, 0-57)
>84 days after fully vaccinated	1-31	0-29	0-15	-0-31	0-69	0-07	0-40	0-20
	(0-20, 2-41)*	(-0-83, 1-41)	(-0-33, 0-62)	(-0-81, 0-19)	(0-07, 1-31)*	(-0-57, 0-70)	(-0-80, 1-60)	(-1-01, 1-41)
Type of SARS-CoV-2 vaccine								
Sinopharm	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Sinovac-CoronaVac	0-71	0-26	0-23	0-05	0-31	0-07	0-31	---
	(0-28, 1-13)**	(-0-07, 0-59)	(-0-07, 0-40)**	(-0-10, 0-20)	(0-06, 0-55)*	(-0-11, 0-25)	(-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	0-93	0-42	0-37	0-67	0-43	0-52	
	(0-74, 1-69)***	(0-43, 1-43)***	(0-22, 0-64)***	(0-15, 0-59)**	(0-41, 0-94)***	(0-14, 0-71)**	(-0-02, 1-06)†	
>28 days	1-28	1-15	0-39	0-36	0-70	0-53	0-62	
	(0-67, 1-89)***	(0-53, 1-77)***	(0-12, 0-66)**	(0-09, 0-63)**	(0-35, 1-04)***	(0-18, 0-88)**	(-0-09, 1-32)†	
Not applicable (partially vaccinated)	-0-71	-0-03	-0-19	-0-06	-0-47	-0-21	-0-28	
	(-1-22, -0-19)**	(0-63, 0-57)	(-0-42, 0-03)†	(-0-32, 0-21)	(-0-76, -0-18)**	(-0-54, 0-13)	(-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.

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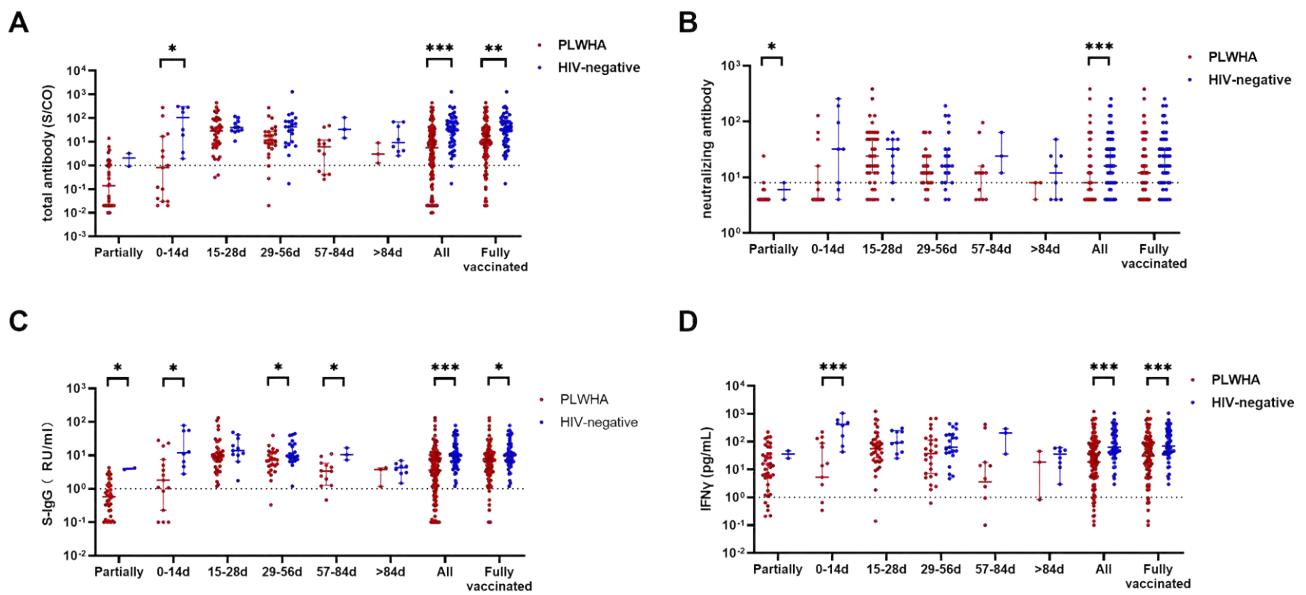


Figure 1. Levels of SARS-CoV-2 neutralizing antibody (A), total antibody (B), S-IgG (C) and T cell specific immune response (D) among HIV-negative individuals and People living with HIV (PLWH) who had received at least one dose of SARS-CoV-2 vaccine

4.10. CoronaVac induces a high immune response in patients with Metabolic Associated Fatty Liver Disease

An article published in the preprint platform SSRN of the british journal The Lancet demonstrated that CoronaVac is safe and immunogenic for people with Metabolic Associated Fatty Liver Disease (MAFLD), being capable to induce the production of IgG antibodies on 100% of the analyzed patients.

Took part of the study 50 people with MAFLD and 50 healthy individuals for controls that received the complete vaccinal scheme of two doses of CoronaVac. The average age was 42 years in the MAFLD group and 40 years in the control group.

One month after the second dose, specific IgG antibodies for the Spike protein were detected on 100% of the individuals of both groups. Six months after the immunization, 94% of the MAFLD patients and 98% of the controls maintained the production of IgG antibodies. Regarding the neutralizing antibodies, 82% of the patients and 90% of the control presented serum conversion.

The immunizer was well tolerated by people with MAFLD and did not have an impact on the status of

the disease. Besides, there were no significant differences in the general incidence of adverse reactions on both groups and all the reported effects were mild.

According to the authors, “our work is the first prospective study of a vaccine against Covid-19 on patients with MAFLD published until this moment. The studies suggest that it is safe and efficient to administer CoronaVac on patients with MAFLD, and this vaccine does not affect the status of the disease. Therefore, the patients with MAFLD must be included in the immunization program against SARS-CoV-2 as a highly vulnerable population with a higher risk of morbidity and mortality”.

The MAFLD is the most frequent hepatic disease in the world, reaching almost 25% of the population. It is associated with metabolic and cardiovascular disorders, such as obesity, insulin resistance, arterial hypertension, dyslipidemia and type 2 diabetes.

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1 **Evaluation of Immune Response and Disease Flares in metabolic-associated fatty**
 2 **liver disease (MAFLD) Patients Following SARS-CoV-2 Vaccination: a**
 3 **prospective study**

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41 **Abbreviations**

42	MAFLD	Metabolic associated fatty liver disease
43	Nab	Neutralizing antibody
44	GMT	Geometric Mean Titers
45	BMI	Body mass index
46	ALT	Alanine aminotransferase
47	AST	Aspartate aminotransferase
48	GGT	γ -glutamyl transpeptidase
49	Alb	Albumin
50	STB	Total bilirubin
51	ALP	Alkaline phosphatase
52	LDL-c	Low-density lipoprotein cholesterol
53	HDL-c	High-density lipoprotein cholesterol
54	TC	Total cholesterol
55	TG	Triglyceride
56	Glu	Glucose
57	HOMA-IR	homeostatic model assessment of insulin resistance
58	hs-CRP	high-sensitive C-reactive protein
59	UA	Urid acids
60	Cr	Creatine

61

62 **Author Contributions**

63 JP Shi conceptualized and supervised the study, QR Zhu, L Shao, J Li, and JP Shi designed the study,
64 QR Zhu, J Gao, JP Gu, L Shen, J Liu, Y Song, XY Gong, YT Chen, J Liao, YN He, SY Zhang
65 collected data, QR Zhu, L Shao, J Li drafted the manuscript, QR Zhu, L Shao, J Li interpreted data, all
66 authors critically reviewed or revised the manuscript and approved the final version of the manuscript.

67

68 **Declaration of Interests**

69 JP Shi reports grants from Project of Key Medical Disciplines of Hangzhou for the Department of
70 infectious & Hepatology. QR Zhu reports grants from the Health and Science and Technology Planning
71 Project of Hangzhou municipal Health Commission, during the conduct of the study. All authors
72 declare no competing interests.

73

74 **Research in context**

75 **Evidence before this study**

76 In patients with metabolic-associated fatty liver disease (MAFLD), existing retrospective data on the
77 risk of adverse outcomes with SARS-CoV-2 infection have been reported. Although the development
78 of SARS-CoV-2 vaccines has shown encouraging safety and efficacy data in many clinical trials,
79 However, concerns have been raised recently about SARS-CoV-2 vaccine responses in patients with
80 MAFLD, such as safety, immunogenicity, and disease flares.

81

82 **Added value of this study**

83 To our knowledge, this is the first prospective study of the safety, immunogenicity, and disease flares
84 of SARS-CoV-2 vaccine in MAFLD populations. We found that SARS-CoV-2 vaccination does not
85 promote disease progression of MAFLD and metabolic comorbidities, and MAFLD patients show a
86 robust immune response after SARS-CoV-2 vaccination in the short term, but this response does not
87 seem to be sustained in the long term. Furthermore, NAFLD fibrosis score was a negatively predictor
88 of neutralizing maintenance.

89

90 **Implications of all the available evidence**

91 Although previous studies reported that metabolic disorders might be significant risk factors of
92 hospitalization and severity in COVID-19 patients, and the effectiveness of vaccination for the
93 MAFLD population is uncertain. Our study showed that a two-dose regimen of CoronaVac vaccination
94 in MAFLD patients was safe and well tolerated. The neutralizing antibody responses appeared to be
95 robust in MAFLD patients who completed vaccination, which conferred 82% protection against
96 COVID-19, and SARS-CoV-2 vaccine did not affect disease flares in MAFLD patients. Therefore,
97 MAFLD patients should be involved in immunization to against SARS-Cov-2. However, liver
98 fibrosis/cirrhosis maybe affect the neutralizing antibody maintenance in MAFLD patients, then future
99 studies should consider booster doses in those with undetectable and suboptimal antibody responses.

100

101

102

103

104 **Summary (300/300 words)**

105 **Background** The ongoing COVID-19 pandemic has led to the focused application of resources toward
106 developing vaccines to prevent COVID-19. However, the efficacy and safety profiles of vaccines
107 against SARS-CoV-2 in patients with metabolic associated fatty liver disease (MAFLD) are still
108 unknown. We aimed to evaluate the safety, tolerability, seroreactivity, and disease flares after SARS-
109 CoV-2 vaccination in MAFLD patients.

110 **Methods** For this prospective observational study, we recruited patients receiving two doses SARS-
111 CoV-2 vaccine (CoronaVac). Neutralizing antibody to the SARS-CoV-2 spike receptor-binding
112 domain and IgG to SARS-COV-2 spike-specific were evaluated on Day 0, Day 28, Day 57, and Day
113 180. All participants with available data were included in the safety and immunogenicity, and disease
114 flares analyses.

115 **Findings** 50 MAFLD patients and 50 healthy controls receiving a 0-28 interval vaccination procedure
116 were enrolled. The seroconversion rates of neutralizing antibodies were 16% in MAFLD group (Log_{10}
117 Geometric Mean Titers (GMT): median 0.783, IQR: 0.719-0.971) and 32% in non-MAFLD group
118 (0.884, IQR: 0.716-1.027) on day 28, and 82% in MAFLD group (1.206, IQR: 1.053-1.467), 90% of
119 non-MAFLD group (1.360, IQR: 1.130-1.464) on day 57, respectively. However, the neutralizing
120 antibody titer in two groups fell below the seropositivity cut-off value on day 180 (MAFLD group
121 0.928, IQR: 0.773-1.057 vs. non-MAFLD group 0.907, IQR: 0.810-1.009). There was no significant
122 difference in the overall incidence of adverse reactions after two-dose vaccinations between two
123 groups. Furthermore, disease flares were not found in MAFLD group after two-dose vaccinations. On

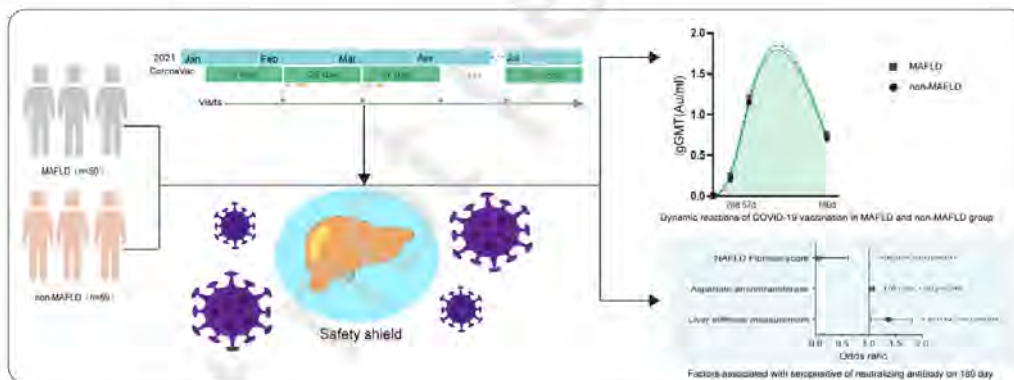
124 multivariable analysis, NAFLD fibrosis score was negatively associated with seropositive of
 125 neutralizing antibody on 180 days (OR 0.03, 95% CI 0.001-0.58, P = 0.022).
 126 **Interpretation** Two-dose regimen of CoronaVac vaccination in MAFLD patients was safe and well
 127 tolerated. MAFLD patients showed a robust immune response after SARS-COV-2 vaccination,
 128 which conferred 82% protection against COVID-19 and vaccination does not affect MAFLD disease
 129 status.

130 **Keywords** COVID-19; SARS-COV-2 vaccination; MAFLD; safety; immunogenicity; disease flares

131 **Funding** Project of Key Medical Disciplines of Hangzhou, the Health and Science and Technology

132 Planning Project of Hangzhou municipal Health Commission (No. A20210205).

133 **Graphical abstract**



134 **Introduction**

135 The persistent COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2
 136 (SARS-CoV-2) has led to high morbidity and mortality worldwide.¹ Metabolic-associated fatty liver
 137 disease (MAFLD), formerly named as non-alcoholic fatty liver disease (NAFLD), is the most common
 138 chronic liver disease, affecting about a quarter of the world's adult population,^{2,3} which often
 139 concurrent with elements of metabolic syndromes, such as diabetes, obesity, or hyperlipidemia are

140 more susceptible to infection and also induce worse outcomes in COVID-19.^{4,5} Early data provided
141 evidence that metabolic syndrome was associated with chronic low-grade inflammation that
142 compromised the immune system and caused microvascular endothelial dysfunction, which was
143 particularly vulnerable to COVID-19 infection and disease progression.⁶⁻⁸ Furthermore, COVID-19
144 infection is reported to associate with disease flares in MAFLD patients.⁹

145 Although encouraging safety and efficacy data of SARS-CoV-2 vaccines has shown in many clinical
146 trials,¹⁰⁻¹² but these studies only included a small number of participants with pre-existing liver disease,
147 such like liver transplantation.^{13,14} Recently, Wang and colleagues demonstrated that COVID-19
148 vaccination is safe and effective in NAFLD patients, while this retrospective study did not set up the
149 control group.¹⁵ However, concerns have been raised recently about SARS-CoV-2 vaccine responses in
150 MAFLD patients, such as safety, immunogenicity, and disease flares. Thus, our study aimed to
151 examine the safety, efficacy, and changes of multiple metabolic indicators of SARS-CoV-2 vaccines in
152 patients with MAFLD.

153

154 **Methods**

155 **Study design and participants**

156 We performed a prospective, observational cohort study that recruited adults (>18 years) receiving
157 SARS-CoV-2 vaccination between 12 January 2021 to 4 February 2021 at the affiliated hospital of
158 Hangzhou Normal University. All participants received two doses of an inactivated vaccine against
159 SARS-CoV-2 (0.5 mL/dose, Sinovac life science, Beijing, China) with a 28-day interval. Hepatic
160 steatosis was defined as a controlled attenuation parameter measurement of 248 dB/m or more.^{16,17}

161 MAFLD diagnosed by hepatic steatosis plus any of the following three metabolic disorders according
162 to the definition proposed by the international expert group¹⁸: 1) overweight/obesity (≥ 23 kg/m²); 2)
163 type-2-diabetes mellitus or 3) metabolic dysregulation. Metabolic dysregulation was defined as the
164 presence of at least two of the following metabolic risk abnormalities: 1) Waist circumference $\geq 90/80$
165 cm in men and women; 2) Blood Pressure $\geq 130/85$ mmHg or use of antihypertensive medications; 3)
166 Triglyceride (TG) ≥ 150 mg/dL or use of lipid-lowering medications; 4) HDL-cholesterol (HDL-c) $<$
167 40/50 mg/dL for male and female or use of lipid-lowering medications; 5) prediabetes (fasting glucose
168 levels 100-125 mg/dL, 2h glucose levels 140-199 mg/dL or HbA1c 5.7%-6.4%); 6) homeostatic model
169 assessment of insulin resistance (HOMA-IR) ≥ 2.5 .¹⁹ We also included immunized non-MAFLD
170 participants without hepatic steatosis, diabetes, and were of normal weight from the same hospital. The
171 study was approved by local Hospital Ethics Committee (2021(E2)-KS-049) and written informed
172 consent was obtained from patients involved before enrolment when data were collected. This trial had
173 been registered in Chinese ClinicalTrials.gov (ChiCTR2100042717).

174

175 **Procedures**

176 Blood samples were captured before vaccination (Day 0), 28 days after the first vaccine dose (Day 28),
177 28 days after the second dose vaccination (Day 57), and 180 days after the first vaccine dose (Day
178 180). Telephone consultations evaluated reactogenicity and safety of each patient within 28 days after
179 injection. Adverse events were graded according to the following scale: grade 1 (mild; does not
180 interfere with activity); grade 2 (moderate; interferes with activity), grade 3 (severe; prevents daily
181 activity), and grade 4 (potentially life-threatening; emergency department visit or hospital admission).

182 ²⁰ Seroreactivity and biochemical indicators were detected at each time point. Neutralizing antibodies
183 (NAb) to the receptor-binding domain (RBD) of SARS-CoV-2 spike protein was detected by iFlash
184 2019-nCoV NAb assay (SHENZHEN YHLO BIOTECH CO., LTD, Shenzhen, China, Cat#C86109),
185 which is a paramagnetic particle chemiluminescent immunoassay (CLIA) for the qualitative detection
186 of SARS-CoV-2 NAb in human serum and plasma using the automated iFlash immunoassay system,
187 and the cut-off value of 10.00 AU/mL for the antibody.²¹ IgG to SARS-COV-2 spike-specific were
188 detected by magnetic particle chemiluminescence immunoassay using SARS-CoV-2 IgG detection kit
189 (Beijing Hotgen Biotech Co., Ltd.). The cut-off was set as 1.00 Au/ml according to the manufacturer's
190 guidelines.

191

192 **Statistical analysis**

193 All participants with available data were included in the safety and immunogenicity analyses. Statistics
194 were computed in IBM SPSS Statistics 26 (Armonk, NY: IBM Corp). The significance threshold for p
195 values was less than 0.05 after correction for multiple comparisons. We used the Pearson χ^2 test or
196 Fisher's exact test for the analysis of categorical outcomes. We calculated Geometric Mean Titers
197 (GMT) and corresponding IQR of the log-transformed antibody titre then used the t-test method to
198 compare the log-transformed antibody titre. Repeated measures ANCOVA, as implemented under the
199 mixed model,²² was applied with change from baseline as the dependent variable, group, time, and the
200 group by time interaction as independent variables. The approximate normality of each outcome and
201 the change score of the outcome was confirmed by examination. Age, sex, BMI, and hypertension were
202 included as covariates to ensure statistical balance was not captured by randomization, and reduce error

203 variance. Logistic regression analysis was used to investigate the association of seroconversion of Nab
204 at day 180 with various metabolic indicators.

205

206 **Role of the funding source**

207 The funder of this study participated in study design, data collection, data analysis, and data
208 interpretation in collaboration with all investigators.

209

210 **Results**

211 **Study design and participants**

212 A total of 164 subjects were screened in the study, and divided into MAFLD group and non-MAFLD
213 group after matching the age. Finally, 60 people were included in the MAFLD group and 60 people in
214 the non-MAFLD group. However, 10 MAFLD patients and 10 non-MAFLD participants did not
215 complete vaccination. Finally, 50 subjects with MAFLD and 50 non-MAFLD subjects were enrolled in
216 our study, respectively (Figure 1). The clinical characteristics of study participants were summarized in
217 Table 1. The mean age of the MAFLD group was 42.10 (9-87), and 39.88(10-50) in the non-MAFLD
218 group. MAFLD patients were more likely to have higher BMI and waist circumference, lower HDL-c
219 and higher level of TG, as well as liver enzyme (alanine aminotransferase (ALT), aspartate
220 aminotransferase (AST), uric acid (UA), high-sensitive C reactive protein (hs-CRP), and HOMA-IR,
221 compared with non-MAFLD patients ($p<0.05$) (Table 1).

222

223 **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 by 5 (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 vaccine²⁴ (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

244 value on day 180. There was no significant difference in the ratio of GMT of Nab from 28 days to 57
245 days and 57 days to 180 days between two groups (Figure 3, appendix p 3).

246 The seroconversion rates of spike-specific IgG were 62% (31/50) in MAFLD group (Log_{10} GMT:
247 median 0.159 [IQR: -0.203, 0.730], 70% (35/50) in non-MAFLD group (0.320 [-0.367, -0.899]) on 28
248 days after the first dose, and 100% in both MAFLD group (1.468 [1.054, 1.928]) and non-MAFLD
249 group (1.643 [0.664, 1.911]) on 28 days after the second dose vaccination. On 180 days after
250 vaccination, seroconversion rates were 94% in MAFLD group (0.851 [0.534, 1.181]) and 98% in non-
251 MAFLD group (0.865 [0.621, 1.187]). Then, we also found no significant difference between the two
252 groups in the ratio of GMT of IgG from 28 days to 57 days and 57 days to 180 days, respectively
253 (Figure 3, appendix p 3).

254

255 **Changes of biochemical indicators**

256 Overall, there was no difference between the two groups in the majority of the absolute value changes
257 of biochemical indicators, such as ALT, AST, γ -glutamyl transpeptidase (γ -GGT), HDL- cholesterol,
258 LDL-cholesterol, total cholesterol, triglyceride, glucose, HOMA-IR, UA, and creatinine after adjusting
259 age, sex, BMI, and hypertension on day 28, day 57, day 180 (Figure 4, appendix p 4,5). In addition,
260 there was also no difference in the majority of biochemical indicators, on day 28, day 57, day 180 in
261 MAFLD patients (appendix p 6,7,8,9).

262

263 **Factors associated with seropositive of neutralizing antibody on 180 days**

264 As shown in Table 2, NAFLD fibrosis score (NFS), liver stiffness measurement, AST, HDL-c,
265 triglyceride, and GMT of neutralizing antibody on 28 days and 57 days were significantly associated
266 with seropositive of neutralizing antibody on 180 days by univariate analyses. Then, on multivariable
267 analysis, the most parsimonious model that optimized prediction only included NFS, which meant that
268 the odds of seropositive of neutralizing antibody at 180 days were higher in those who with lower NFS
269 (OR 0.03, 95% CI 0.001, 0.58) (Table 2).

270

271 **Discussion**

272 Previous studies reported that the incidence of COVID-19 was higher in MAFLD group than in non-
273 MAFLD group.^{25,26} In addition, metabolic disorders might also be significant risk factors of
274 hospitalization and severity in COVID-19 patients.^{27,28} Therefore, it's urgently needed to explore the
275 SARS-CoV-2 vaccine responses in MAFLD patients as those patients may be uniquely susceptible to
276 COVID-19 infection and disease progression. To the best of our knowledge, this is the first prospective
277 report of the safety and immunogenicity of SARS-CoV-2 vaccine in MAFLD populations. Our study
278 indicated that there was no significant difference in the overall incidence of adverse reactions after two-
279 dose vaccinations between two groups, and SARS-CoV-2 vaccine did not affect the biochemical
280 indicators in MAFLD patients. Furthermore, we detected that NAFLD fibrosis score was inversely
281 associated with seropositive of neutralizing antibody on 180 days.
282 Similar to the general population²⁹, side effects related to the SARS-CoV-2 vaccine in MAFLD patients
283 were mild and self-limiting, and the most common symptom was injection-site pain, followed by
284 fatigue, dizziness, and diarrhea. No serious adverse events were reported in MAFLD patients. Our

285 results indicated that a two-dose regimen of 3 ug of inactivated CoronaVac vaccine administered 28
286 days apart to MAFLD patients was safe and well tolerated. Furthermore, we did not find changes of
287 biochemical indicators, especially ALT, AST, γ -GGT after vaccinations in MAFLD patients, which
288 means that CoronaVac vaccination might not affect the disease status and also prove the safety of
289 SARS-CoV-2 vaccine in special population.

290 Vaccine immunogenicity is broadly assumed to require neutralizing antibodies, although its protection
291 role against COVID-19 remains incompletely defined. Our results showed that two-dose CoronaVac
292 induced neutralizing antibody and spike-specific IgG in MAFLD patients still comparable, which was
293 consistent with previous study.²⁴ Similar to the study performed by Wang et al.,²⁹ CoronaVac elicited a
294 high immune response in our cohort in the short term, with 82% vaccine efficacy in MAFLD group at
295 28 days after two-dose vaccinations. However, the GMT of Nab declined to below the positive cutoff
296 titer after 6 months of vaccination in our cohort, which was also consistent with the results of Pan et
297 al.'s study using the same vaccine.³⁰ Pan et al.'s study also found that a third dose vaccination, given at
298 an interval of 6-8 months after the second dose could lead to a significant rebound in antibody levels,
299 which indicating that booster vaccination may be necessary.

300

301 On multivariable logistic regression analysis, few variables were associated with seroconversion rates
302 of neutralizing antibodies after vaccination and liver steatosis, abnormal liver function, and elevated
303 BMI were not associated with the poor antibodies responses, which provides encouraging evidence for
304 MAFLD patients, who should be more actively involved in SARS-CoV-2 immunization. However,
305 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

327 to be sustained in the long term is a major concern and liver fibrosis/cirrhosis may affect the
 328 neutralizing antibody maintenance in MAFLD patients, then a third dose could be necessary to boost
 329 immunity. However, future studies should consider booster doses in those with undetectable and
 330 suboptimal antibody responses.

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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,

creatinine. * $p < 0.05$.

Table 1: Patient baseline characteristics, comorbidities by MAFLD stature

Characteristics	MAFLD group	non-MAFLD	p
	(N=50)	group (N=50)	
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 (%), ^arepresent 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)	<i>p</i>	B	OR (95% CI)	<i>p</i>
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.

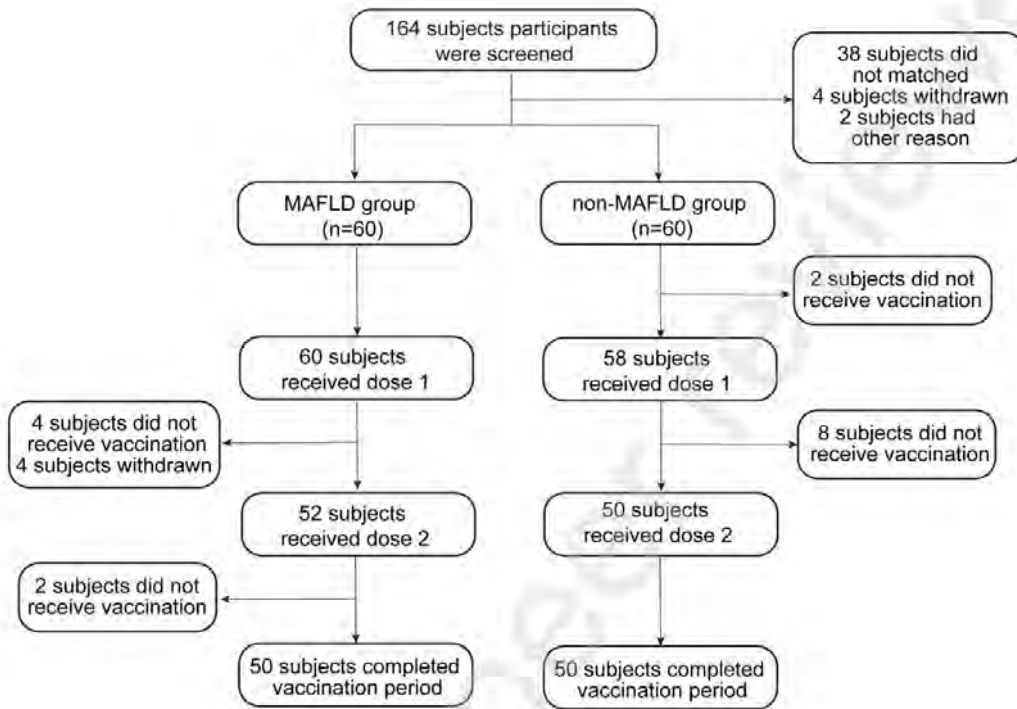


Figure 1: Screening and vaccine administration

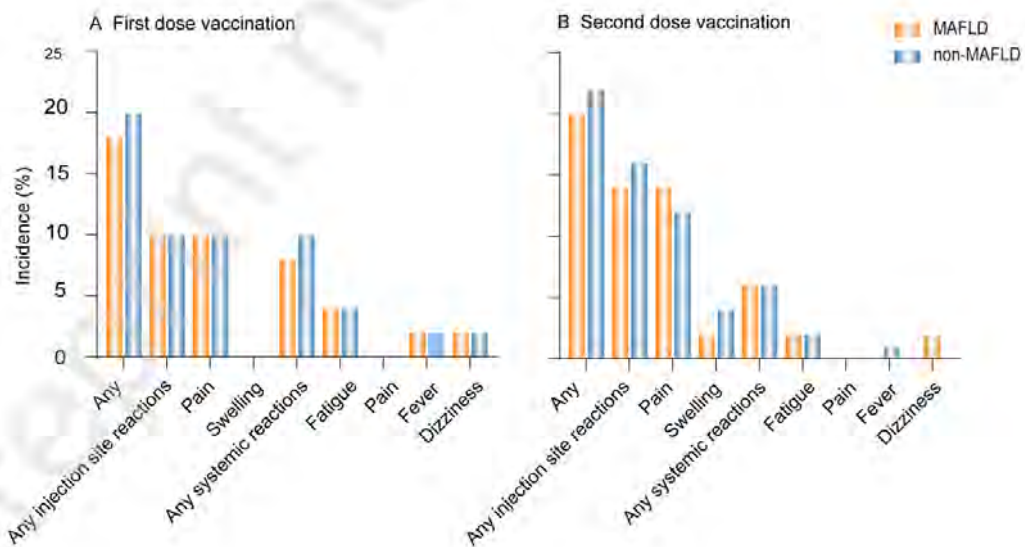


Figure 2: Adverse reactions of COVID-19 vaccination in MAFLD and non-MAFLD group

Incidence of adverse reactions reported within 28 days after the first dose vaccination (A) and the second dose vaccination (B) between the two groups.

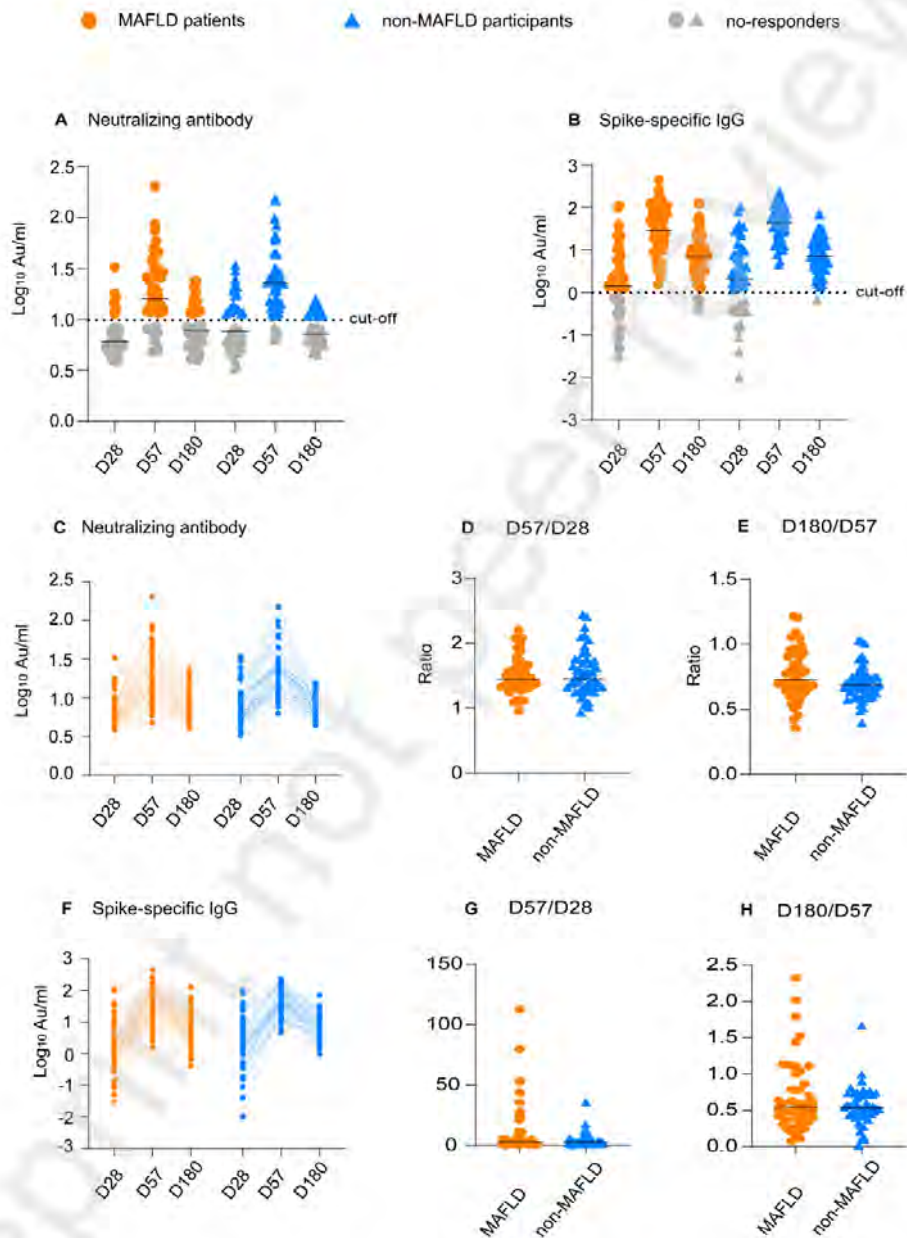


Figure 3: Serological response to COVID-19 vaccine

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).

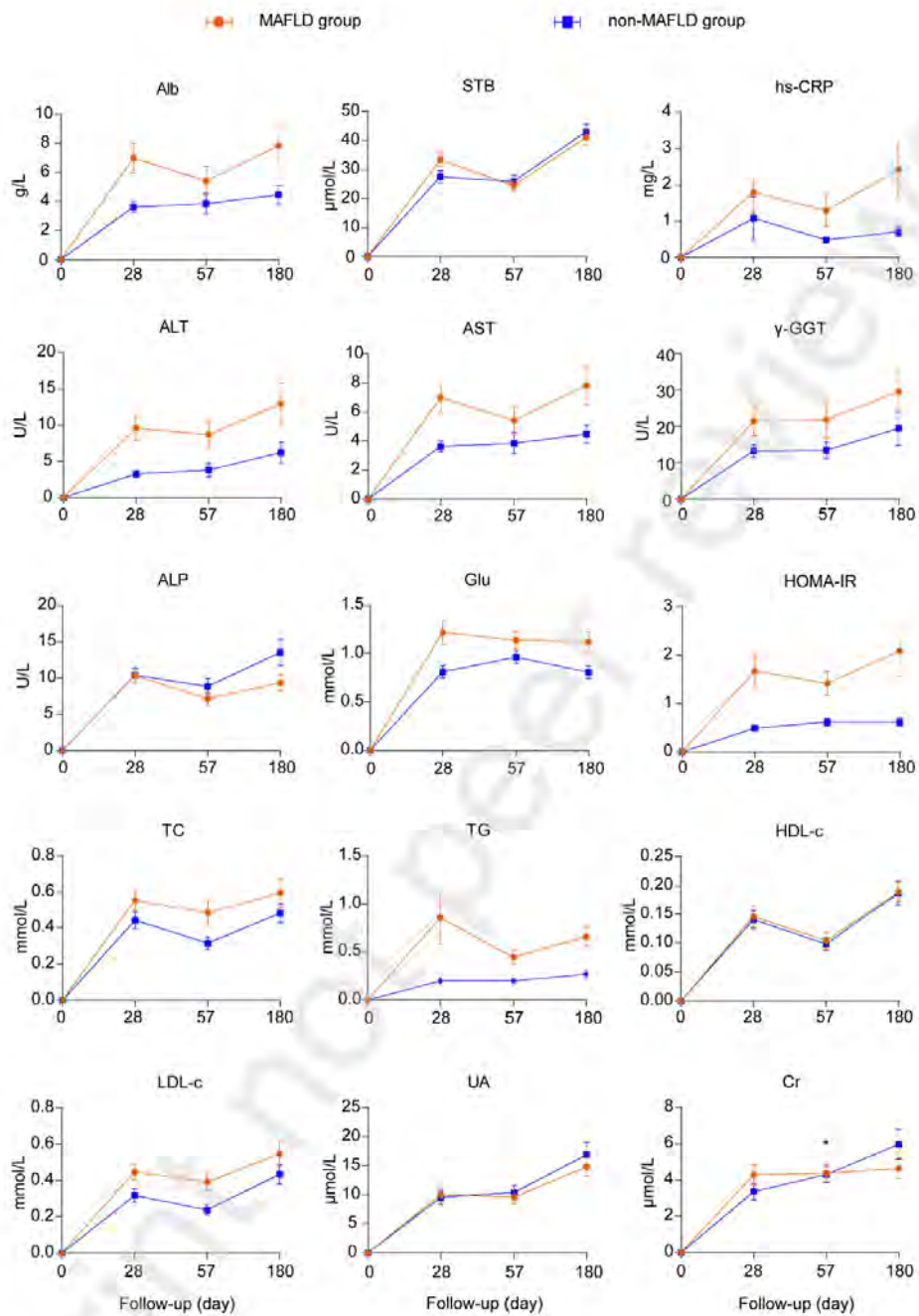


Figure 4: Dynamic absolute changes of biochemical indicators at different timepoints

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. Study proves the efficacy of CoronaVac against Covid-19 on patients with cancer

A study made in Turkey and published in the Future Oncology journal demonstrated that CoronaVac, a vaccine from Butantan and the Chinese pharmaceutical Sinovac, has efficacy and generates protection against Covid-19 on patients in treatment against cancer. Two weeks after the application of the second dose of the immunizer, there was seroconversion (which means, production of antibodies) in 63,8% of the participants.

The immunogenicity rate reached 100% on patients that receive only monoclonal antibodies or immunotherapy as medication. Besides, none of the patients presented infection by Covid-19 on an average monitoring of 85 days after completing the vaccinal scheme. The gap between the application of both doses of CoronaVac was 28 days.

This is the first study published that analyzes the efficacy of CoronaVac on oncological patients. The conclusions are in the article "Immunogenicity and safety of the CoronaVac vaccine in patients with cancer receiving active systemic therapy", written by researchers that work in seven hospitals and two universities of Ankara.

The research was conducted between January and April of 2021 with 47 patients with solid tumors. They presented, in order of frequency, colorectal cancer, breast cancer, lung cancer, genitourinary, gastric, pancreas, gynecological, of the biliary tract and the central nervous system. Most of the patients

were diagnosed with stage IV of the disease and received palliative systemic treatment. The average age of the patients was 73 years, and none of them had previous contact with the SARS-CoV-2 virus.

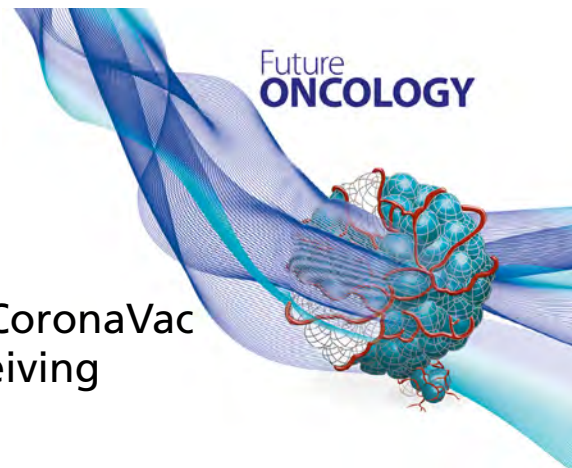
Besides the immunogenicity, the study analyzed the safety of the vaccine. After receiving the first and the second dose of CoronaVac, the rates of adverse effects of any level among the 47 analyzed patients were 18,9% and 23,1% respectively. No severe adverse effects were observed.

The results of the Turkish research join the other recently disclosed articles that also confirm the efficacy of CoronaVac on immunosuppressed people, a public that has the most difficulty in the immunological defense of the organism.

A research of the Clinical Hospital from University of Sao Paulo Medical School demonstrated that patients with autoimmune rheumatologic diseases presented an increase of 70,4% on the levels of antibodies against the SARS-CoV-2 virus two weeks after receiving the second dose of CoronaVac. Besides, the scientists of the Federal University of Sao Paulo and of the Blood Center of Ribeirão Preto from the University of Sao Paulo concluded that 43% of the kidney transplanted patients that were analyzed generated antibodies against Covid-19 15 days after receiving the second dose of the vaccine.

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Research Article

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Immunogenicity and safety of the CoronaVac vaccine in patients with cancer receiving active systemic therapy

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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.

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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

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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	Docetaxel	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- μ g 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 immunotherapy, 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.

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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.

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4.12. CoronaVac increases antibodies against Covid-19 by 70% in immunosuppressed patients, says study

Patients with autoimmune rheumatologic diseases showed an increase of 70.4% in the level of antibodies against the SARS-CoV-2 virus two weeks after receiving the second dose of CoronaVac, the vaccine against Covid-19 from Butantan and the Chinese pharmaceutical company Sinovac. In addition to increasing the seroconversion of immunosuppressed patients, CoronaVac also raised the amount of neutralizing antibodies by 56.3%.

The conclusions are from a study conducted by the Clinical Hospital of the Medical School of the University of São Paulo (HCFMUSP), with 910 people, and are described in the article “Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial”, published in the scientific journal Nature Medicine.

The result is extremely positive because it shows that CoronaVac is not only well accepted by the organism of immunosuppressed patients (who have more difficulty in producing antibodies), as well as generates a high level of defense and neutralizing antibodies. The research shows that not only

CoronaVac is safe in this audience, but also effective.

“This is the largest study ever conducted in the world with immunosuppressed patients of rheumatologic diseases” says the clinical director of HCFMUSP, Eloisa Bonfá. “The increase in antibody levels is very relevant and shows that CoronaVac conferred an important protection among immunosuppressed patients” she adds.

Another fact that attests the safety of CoronaVac is the absence of adverse reactions in the vaccinees. “We didn’t have any cases of severe or moderate side effects among the patients, even though this could be expected among immunosuppressed patients. We only had mild side effects. CoronaVac is a highly safe vaccine”, Eloisa points out.

According to the hospital director, the 910 immunosuppressed patients participating in the study were vaccinated with two doses. Shortly after the second dose, when the antibodies were still being produced, there were 33 cases of Covid-19; 40 days later, that number had dropped to six cases.

Why is this result so relevant?

The seroconversion result (capacity to produce antibodies) of CoronaVac in immunosuppressed patients from HCFMUSP is surprising, especially when compared with the control group, composed of people without immune deficiencies. The level of defense antibodies generated in the immunosuppressed patients was 70.4%, while in the control group it was 95%; and the level of neutralizing antibodies was 56.3% in the immunosuppressed patients, and 79.3% in the control group.

People with autoimmune rheumatic diseases are usually treated with corticosteroids combined with immunosuppressants. In other words, their treatment usually involves medications that act to suppress the immune system, preventing it from acting in a way that aggravates the autoimmune disease.

The consequence is that immunosuppressed people have a lower capacity to produce

antibodies. Therefore, their organisms are more susceptible to contract infectious diseases, as in the case of Covid-19, and evolve to severe cases. Before the HCFMUSP research, this public was prevented from taking the vaccine and could only count on measures still under development, such as the anti-Covid serum.

Autoimmune rheumatologic diseases comprise several syndromes, such as autoimmune myositis, eosinophilic fasciitis, mixed connective tissue disease, relapsing polychondritis, Sjögren's syndrome, systemic lupus erythematosus and scleroderma.

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Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial

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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 $\geq 15\%$ 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 world¹. 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 variant³, which is considered to be 2.5-fold more contagious than the original strain⁴ and possibly associated with a higher risk for hospitalization and intensive care unit admission in patients younger than 60 years of age⁵. 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 Turkey⁷, 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 refrigerated⁸, 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 trial¹⁰ 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 population¹². 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

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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 shedding²⁵, reduced SARS-CoV-2 virus clearance and enhanced viral genomic evolution²⁶, 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 (CoronaRheum 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 ARD²⁷.

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 (<i>n</i> = 910)	CG (<i>n</i> = 182)	<i>P</i> 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			
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 $\geq 30\%$ (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

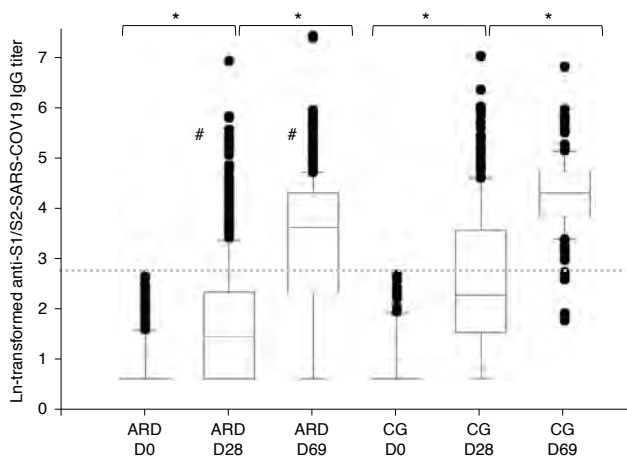


Fig. 1 | Anti-SARS-CoV-2 S1/S2 IgG titers of patients with ARD and subjects in CG at D0, D28 and D69. Box plots show the distribution of ln-transformed IgG titers over time. Data for each group (ARD, $n = 859$ and CG, $n = 179$) are presented at each time point as box plots: central values within boxes 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” = $Q3 + 1.5 \times IQR$) and minimum (lower “calculated value” = $Q1 - 1.5 \times IQR$). 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 $\text{ml}^{-1} = 2.71$ 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 phase3 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 population¹⁰, 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 thrombosis²⁸, a rare complication reported for some of the new COVID-19 vaccines²⁹, and autoimmune/autoinflammatory manifestations, a problem with adjuvanted vaccines in this already predisposed population³⁰. Similar to previous results from CoronaVac trials in healthy populations³¹, 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 CoronaVac¹¹, 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 time-frame, 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 population¹⁷ and in a study with patients predominantly using cytokine inhibitors and with limited representation of systemic diseases¹⁶. 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 immunogenicity³⁸.

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 drugs¹⁸. 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 patients¹⁹. We also found a detrimental effect of TNFi therapy solely on anti-S1/S2 IgG response, contrasting with a recent study of patients with ARD¹⁶. 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 VOC⁴¹.

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>.

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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 Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São 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: RA⁴², SLE⁴³, axSpA⁴⁴, PsA⁴⁵, primary vasculitis^{46,47}, pSS⁴⁸, SS⁴⁹, IIM⁵⁰ and PAPS⁵¹.

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 diseases; 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 μ g 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 ($\geq 15.0 \text{ UA ml}^{-1}$) 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 ($< 3.8 \text{ UA ml}^{-1}$). 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 $\geq 30\%$) or "negative" (inhibition $< 30\%$), as suggested by the manufacturer⁵². 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 definition⁵³.

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 test⁵⁶. 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 $\Delta 106-108$, 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 $\Delta 3675-3677$ and Spike $\Delta 69-70$ primer) and facilitates differentiation of Alpha VOC from Beta and Gamma VOC, and from ancestral variants⁵⁸. 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)^{59,60}. 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-LSK109 Kit (Oxford Nanopore Technologies). Sequencing libraries were loaded onto an R9.4.1 flow-cell (Oxford Nanopore Technologies) and sequenced using MinKNOW v2.10.3 (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 ARD³⁶. 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 vaccine²⁸, 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 Clínicas da Universidade de São 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.).

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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

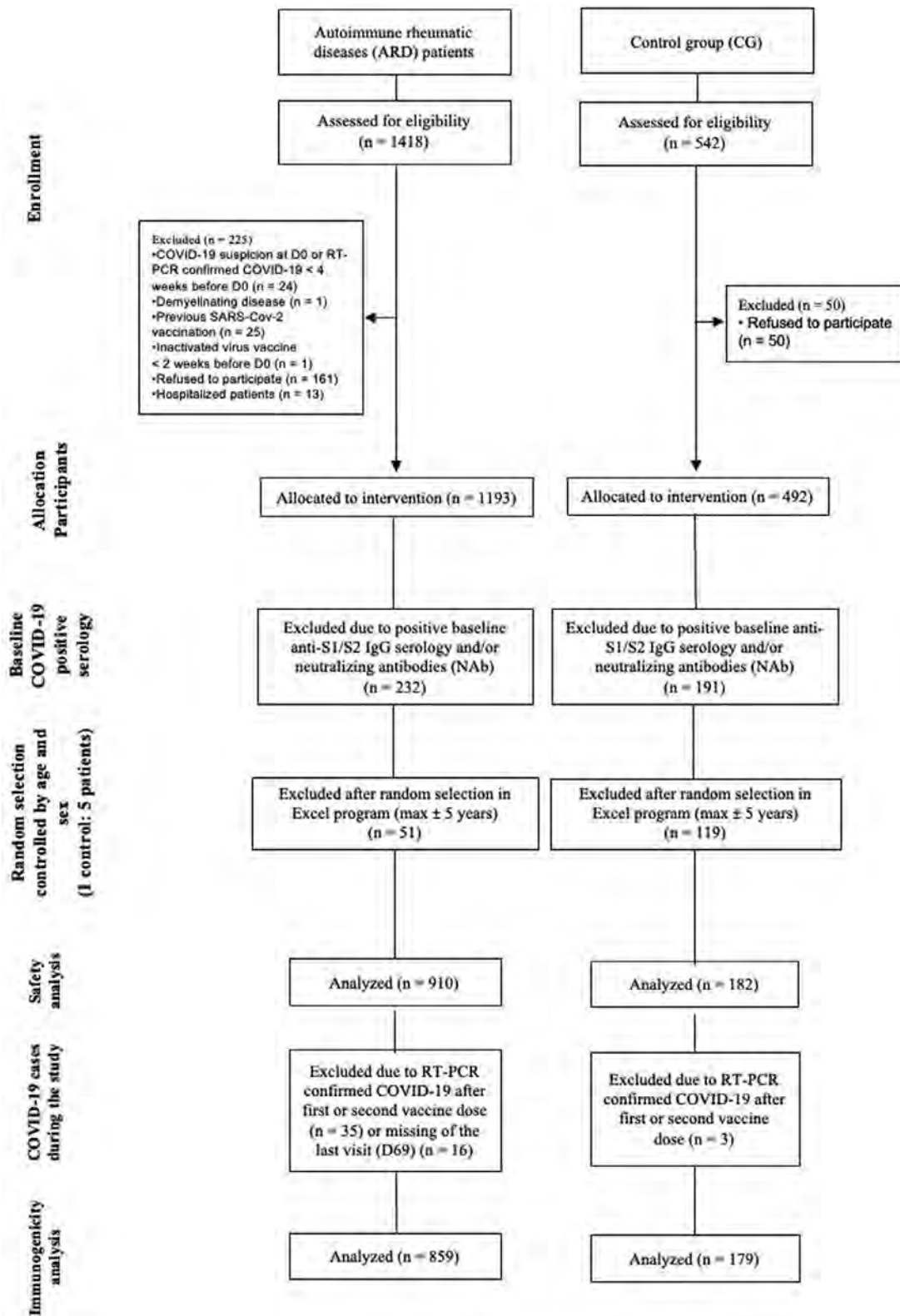
Extended data is available for this paper at <https://doi.org/10.1038/s41591-021-01469-5>.

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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.

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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)

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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.).

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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 patients ³⁵ . 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.

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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 immunogenicity 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. Immunogenicity 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

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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 mean titres (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 $\geq 30\%$ 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 helps to improve the immunity on transplanted patients, affirms study from Unifesp and USP

A study made by researchers from Instituto Butantan, the Federal University of Sao Paulo (Unifesp) and the Blood Center of Ribeirão Preto from the University of Sao Paulo (USP) demonstrated that 43% of the patients with transplanted kidney generated antibodies against Covid-19 15 days after receiving the second dose of CoronaVac (which means, they presented seroconversion). The result indicates that the vaccine from Butantan and the chinese pharmaceutical Sinovac have an effect on that public that is slightly superior to other two immunizers, that use the technology of mRNA and that generated antibodies in about 30% of the cases, according to studies.

This data shows the importance of the vaccine for all the immunosuppressed people that, like patients with transplants and people with autoimmune diseases, have a higher difficulty in the immunological defense of the organism.

“All vaccines have less efficacy on those that are transplanted

because of the use of medications against the rejection of the transplant. This happens with all the immunizers against hepatitis B, influenza, pneumonia and also with the vaccine against the Coronavirus”, explains the main author of the article and full professor of the transplanting area from the Paulista Medicine School of Unifesp, José Medina.

The work was developed in the Kidney Hospital and its preliminary results were disclosed in an article at the Transplantation journal, the main worldwide publication in the transplants area. The research was conducted between 20th and 28th of March 2021, with 3.354 patients with kidney transplant between 30 and 69 years of age, that have done the transplant over 30 days before, did not have previous case of Covid-19 and completed the vaccinal scheme of two doses from CoronaVac with a gap of 28 days.

“Since the number of transplanted people is small for a general population, as soon as the majority

of people get vaccinated the circulation of the coronavirus will decrease, protecting as well the transplanted people”, affirms Medina. The seroconversion rates among the kidney transplanted patients after the first and second dose of CoronaVac alerts for the need of keeping the individual protection measures, like wearing a mask, avoiding agglomerations and always sanitizing the hands.

The receivers of kidney transplant were included in the national calendar of vaccination against Covid-19 in the priority group with comorbidities in April 2021, because of the high rates of mortality associated with SARS-CoV-2 in that population (up to 30%).

The conclusions of this study adds to another research made by the Clinical Hospital of USP, where 1.000 patients with rheumatological

diseases (also immunosuppressed) were vaccinated with CoronaVac. The immunization generated a moderate immune response on the patients: the monitoring before and after the vaccine presented 33 cases of Covid-19 before the vaccination and only six cases after the immunization.

The efficacy of CoronaVac was proved in Brazil through a study with 13.060 volunteers, all healthcare workers, a population that is highly exposed to Covid-19. The results of the clinical trial of phase 3 demonstrated that the general efficacy of the immunizer may reach 62,3% when the gap between the first and second dose is from 21 to 28 days. The data were disclosed in the preprint platform SSRN.

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Clinical Impact, Reactogenicity, and Immunogenicity After the First CoronaVac Dose in Kidney Transplant Recipients

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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.

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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 2nd dose, 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 (3 μ g 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.

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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)			
Score throat	65 (2)			
Fever	39 (1)			
<hr/>				
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 AU/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%		

^aP = 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. It is effective in the elderly

5.1. CoronaVac is safe for the elderly and has a low rate of adverse events, research shows

A study carried out in Hong Kong to assess the risk of adverse events in the elderly immunized with CoronaVac once again proved the safety of the vaccine, with 79.9 mild adverse events occurring per 100,000 elderly vaccinated. The paper was published in The Lancet Health Longevity journal and conducted by researchers at the School of Medicine of the University of Hong Kong.

Of the 1.25 million people in Hong Kong who received at least one dose of CoronaVac between February 2021 and January 2022, 622,000 (49.6%) were at least 60 years old – 293,000 (47.9%) men and 329,000 (52.9%) women.

Among the 622,000 elderly people investigated, 126,000 (20.4%) received one dose of CoronaVac, 384,000 (61.7%) received two doses and 111,000 (17.9%) received three doses of the vaccine. The data

are from the Government of Hong Kong's Department of Health.

According to the researchers, the incidence of adverse events in the 21 days after vaccination with CoronaVac was low. Among all categories of adverse events of special interest, only 79.9 cases were recorded, all mild, for each group of 100,000 immunized.

“In comparison with the risk of mortality and complications from COVID-19 in the elderly, the benefits of vaccination with CoronaVac outweigh the risks. Our results are consistent with the findings of previous studies with inactivated vaccines, showing its safety among other risk groups, such as the immunocompromised and people with chronic hepatitis B virus infection,” the authors state.

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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



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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 1253497 individuals who received at least one dose of CoronaVac during the study period, 622317 (49.6%) were aged at least 60 years and were included in the analysis. Our analysis sample received 1229423 doses of CoronaVac and had a mean age of 70.40 years (SD 8.10). 293086 (47.1%) of 622317 participants were men and 329231 (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.

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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

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For the Chinese translation of the abstract see [Online](#) for appendix 1

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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 Turkey⁹ 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 study¹³ 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.

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}

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.

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).

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

For the study protocol see <https://www.hkcare.hku.hk/>

For the clinical management system of the Hospital Authority see <https://www.ha.org.hk/>

See Online for appendix 2

For the Deaths Registry see <https://www.immd.gov.hk/eng/services/birth-death-marriage-registration.html>

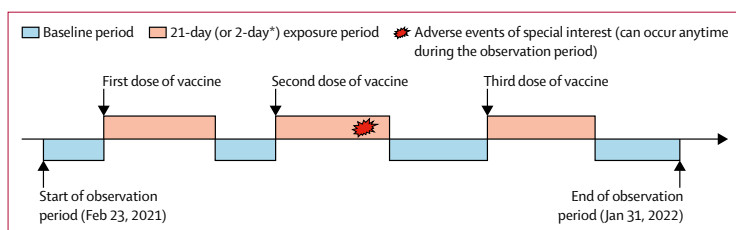


Figure 1: Observation timeline of a hypothetical patient in the self-controlled case series
 *The exposure period was 2 days for anaphylaxis.

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 Furthermore, 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 1253497 individuals who received CoronaVac in Hong Kong between Feb 23, 2021, and Jan 31, 2022, 622317 (49.6%) were aged at least 60 years and were included in the analysis. Overall, 1229423 doses of CoronaVac were administered to these 622317 people within the study period, among whom 126736 (20.4%) had one dose, 384056 (61.7%) had two doses, and 111525 (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

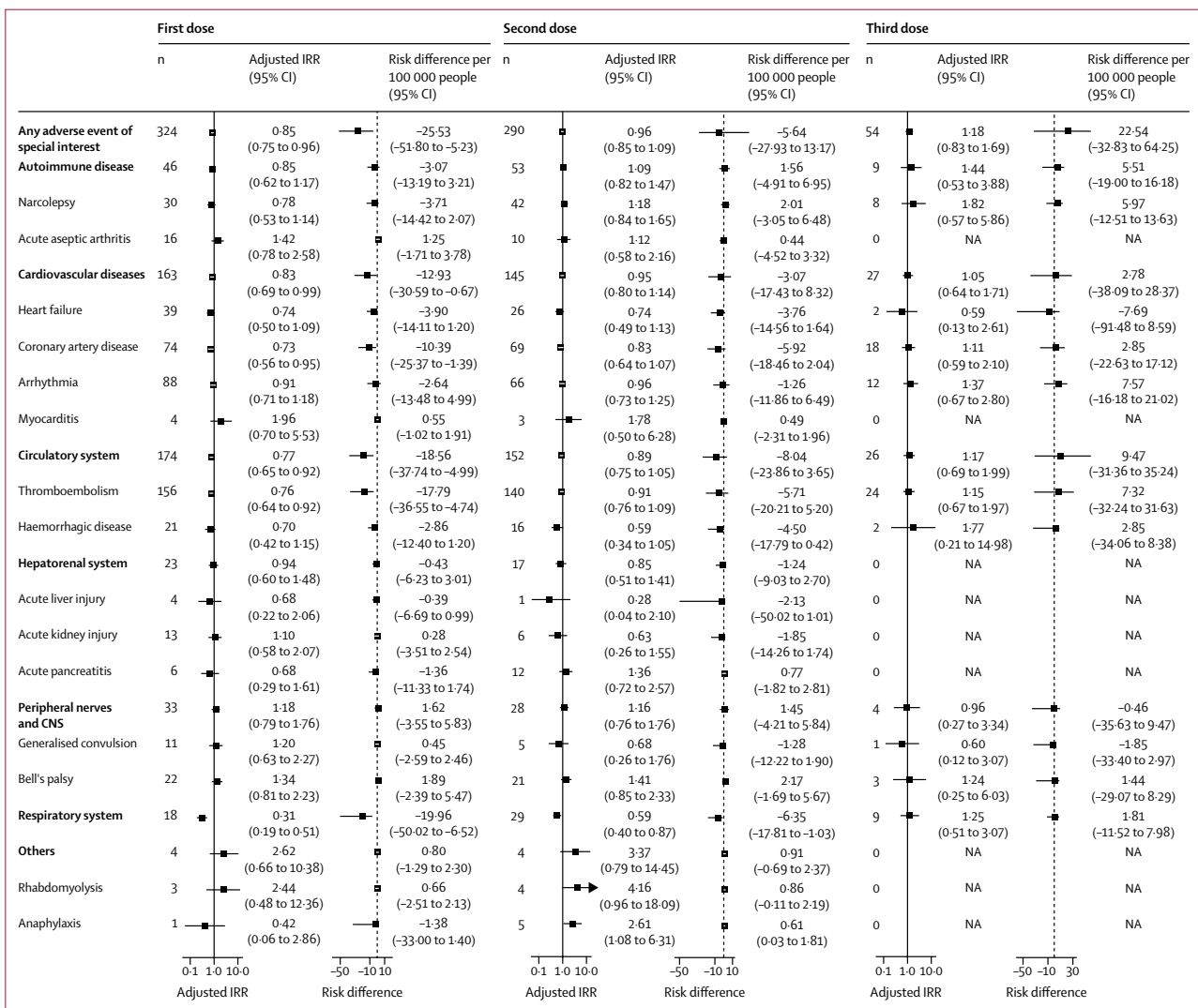


Figure 2: Adjusted IRRs and risk differences of adverse events of special interest within 21 days of CoronaVac vaccination
Adjusted IRRs were obtained from conditional Poisson regression adjusted for seasonal effects in our self-controlled case series analysis. The dashed line represents a risk difference 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 patients⁴ 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 vaccination²⁹ 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 study⁴⁸ 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 study⁴¹ 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

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Data sharing

Data will not be made available to others because the data custodians have not given permission.

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CoronaVac

What science proves

5.2. CoronaVac reduced severe cases and death from Covid-19 in the elderly, Colombian study states

A study carried out in Colombia with about 700,000 participants showed once again that administering CoronaVac to people over 60 years of age substantially reduces severe cases and death from Covid-19. The study was published in *The Lancet Regional Health – Americas* and conducted by researchers from the National University of Colombia and the Colombian universities of Sinú, Cartagena and de la Costa, as well as other institutions such as the University of São Paulo.

Between March and August 2021, scientists followed 720,000 people with an average age of 68. Among the volunteers, 76,700 had been vaccinated with CoronaVac, 56,000 had received another vaccine, and 539,000 had not yet been immunized.

Among unvaccinated individuals, there were 21,500 symptomatic cases of Covid-19, 2,874 hospitalizations, 1,061 admissions to Intensive Care Units (ICU) and 1,329 deaths. In those vaccinated with CoronaVac, the immunizing agent reduced the risk of hospitalization and death from Covid-19 by more than half.

The authors draw attention to another study conducted in Colombia with about 3 million

people, which showed an even greater effectiveness of the vaccine to prevent deaths in the elderly (72.1%). According to research published in *The Lancet Healthy Longevity*, less than 1% of elderly people who took CoronaVac died or needed to be hospitalized as a result of Covid-19.

According to the researchers, the difference in results can be explained by the lower statistical power of the current sample (720 thousand against 3 million) and also by the socioeconomic background of the volunteers. While the previous work was done with the entire Colombian population, the current research only included participants from the north of the country with low socioeconomic status – a group that is more at risk of being infected by SARS-CoV-2 and has less access to health services.

The relationship between Covid-19 and social vulnerability has already been demonstrated in different countries. More recently, a study by the University of São Paulo carried out in the Amazon showed that children who went hungry were 76% more likely to have symptomatic Covid-19.

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Effectiveness of CoronaVac and BNT162b2 COVID-19 mass vaccination in Colombia: A population-based cohort study

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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.

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Keywords: Vaccine effectiveness; Covid-19; Mortality; Vaccines

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Introduction

The SARS-CoV-2 virus first appeared in Wuhan, China in late-2019, causing a cluster of cases of COVID-19

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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, Córdoba, 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 2022.^{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.

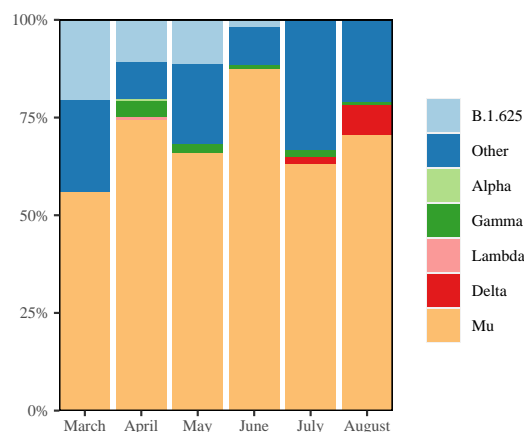


Figure 1. Monthly distribution of identified variants of SARS-CoV-2 in Colombia during the study period.

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.

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 censored 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 dividing 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 3.2.11).

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, -53.2 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 Montería (capital of Córdoba) 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% CI, 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 socioeconomic status, which is a driver of infection and adverse outcomes. Studies from United States,^{15,16} Germany,¹⁷ UK,¹⁸ Chile,¹⁹ and Colombia²⁰ 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 study²⁰ 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 cases⁴ 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 samples¹² (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 significantly 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-Cacedo 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.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.lana.2022.100296.

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5.3. Boosting CoronaVac in the elderly raises antibody levels 43 times, says Chinese study

A Chinese study published in the high-impact journal *Nature Communications* showed an increase in antibody levels after the third dose of CoronaVac, both in the elderly and in younger adults. The research was carried out by Fudan University, the Centers for Disease Control and Prevention in Hebei and Jiangsu provinces, and pharmaceutical company Sinovac.

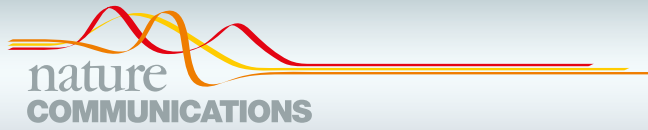
Scientists conducted two phase 2 clinical trials to evaluate the immunogenicity and safety of three doses of CoronaVac in 600 adults, ages 18 to 59, and 350 older adults, age 60 and older. Six months after the second dose, drops in antibody titers were observed, as with all vaccines currently available against Covid-19.

In the adult group, the booster dose raised antibody levels 33-fold with

the 6 µg dose (to 230.9) and 21-fold with the 3 µg dose (to 143.3). The same pattern was observed in the elderly volunteers: the third dose increased the antibodies 43-fold (178.9, with 6 µg of the immunizing agent) and 46-fold (158.5, with 3 µg).

Furthermore, after the third dose, neutralizing antibodies were maintained longer than observed after the second. “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. 6µg) could be considered for booster immunisation programmes,” the researchers state.

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Six-month follow-up of a booster dose of CoronaVac in two single-centre phase 2 clinical trials

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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.

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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 Organisation⁶, 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

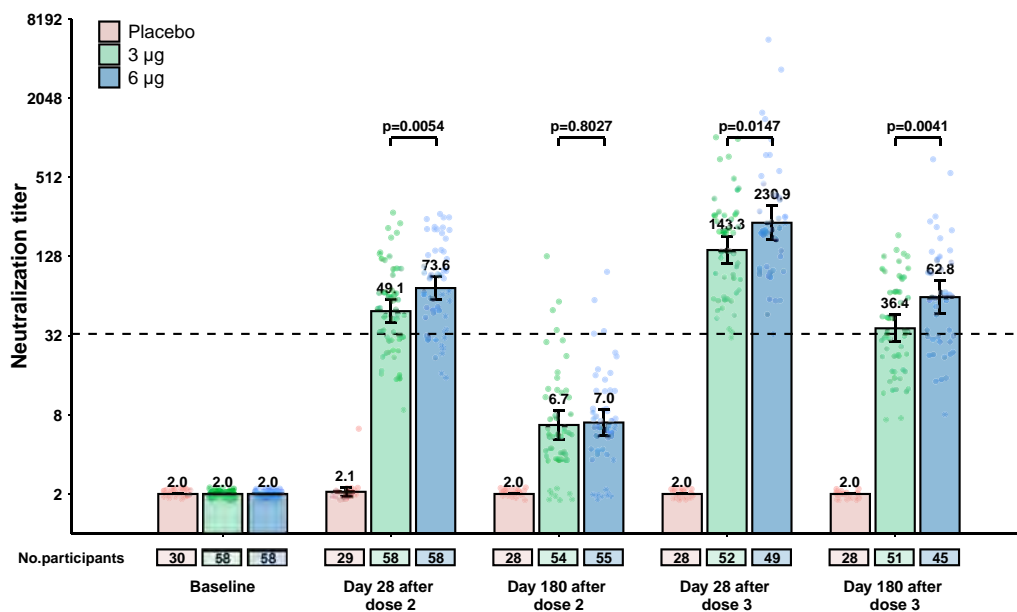


Fig. 1 Neutralising antibody levels to ancestral SARS-CoV-2 in cohort 2b-28d-8m (adults aged 18–59 years old). The number of participants for each group (placebo group, pink; 3 µg group, green; 6 µg group, blue) at each visit included in the analysis is provided below the bars. Dots are reciprocal neutralising 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 previously¹³. 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

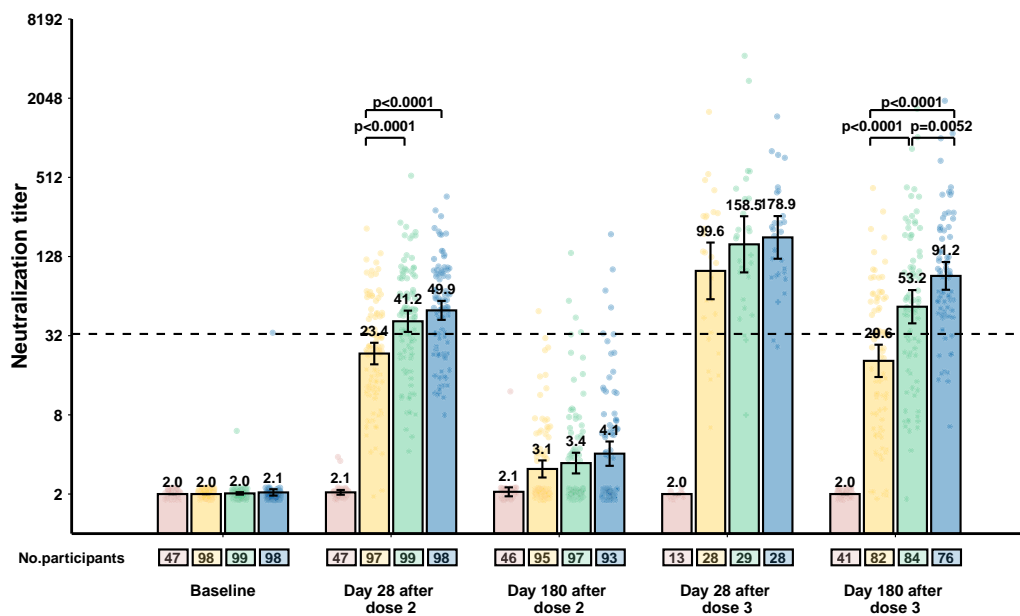


Fig. 2 Neutralising antibody levels to ancestral SARS-CoV-2 in cohort 3-28d-8m (adults aged 60 years and older). The number of participants for each group (placebo group, pink; 1.5 µg group, yellow; 3 µg group, green; 6 µg group, blue) at each visit included in the analysis is provided below the bars. 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).

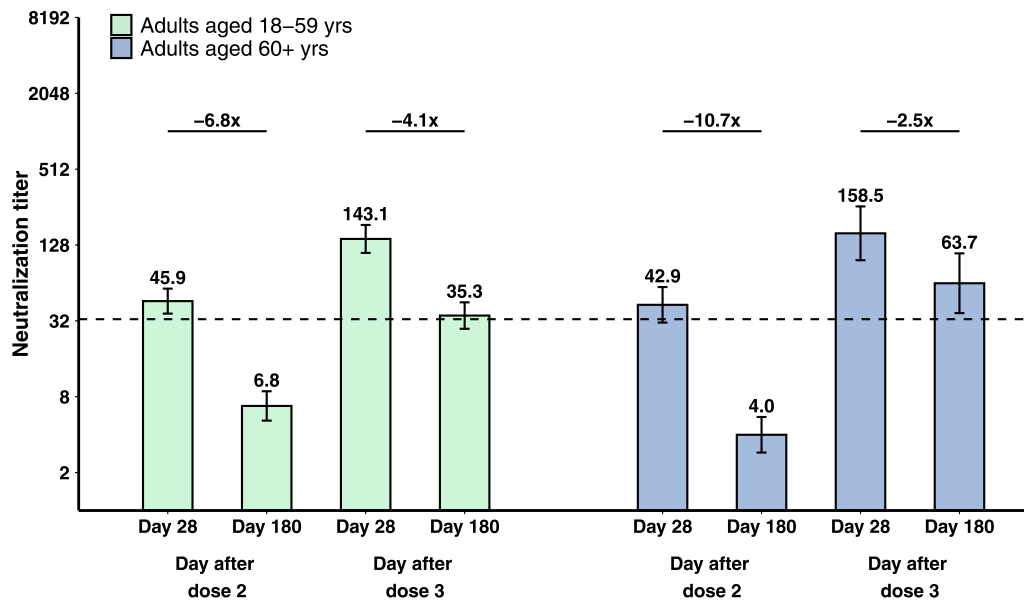


Fig. 3 Decline in neutralising antibodies to ancestral SARS-CoV-2 in 3 µg groups in cohort 2b-28d-8m and cohort 3-28d-8m. The number of participants with paired samples for adults aged 18–59 (green) and adults aged 60 and over (blue) was 49 and 29, respectively. Numbers above the bars are geometric mean 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* = 0.0187, 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 BNT162b2⁴ 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 adults¹⁴. 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 recipients¹⁵. Age did not appear to compromise antibody response, even after accounting for severity among COVID-19 patients¹⁶. 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 strains¹⁷. However, for individuals vaccinated with CoronaVac, the average reduction against Delta was 9.2-fold compared with the prototype strain using authentic virus neutralisation assay¹⁸. 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 test¹⁹. Another study showed a higher fold reduction of 41.4 for the Omicron variant among individuals with previous infection or vaccination²⁰.

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 Delta²¹. 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 concern⁹. 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 vaccination²², 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 induced²⁵. 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 reported¹¹. 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 previously¹¹, 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 previously¹¹. 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 colleagues¹⁰, 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.

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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>.

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5.4. CoronaVac protects the elderly against severe Covid-19, Chinese study shows

A Chinese study carried out in Nanjing once again showed the efficacy of CoronaVac in preventing the elderly from developing the severe type of Covid-19, demonstrating that the vaccine is able to protect even against the delta variant of SARS-CoV-2. The study was published in the journal *Aging* and conducted by researchers at Nanjing Medical University Hospital in China.

The scientists selected 181 patients aged 60 and over who were infected with the delta variant, admitted between July and September 2021 at the Nanjing Public Health Center. Of these, 111 had comorbidities. The volunteers were split into three groups: group A, with 113 participants that had not been immunized; group B, with 46 that had only taken the first dose of CoronaVac; and group C, with 22 that received the complete two-dose schedule.

Among the patients, 145 were classified as moderate cases of Covid-19, 21 progressed to the severe form and 15 were critical cases, which resulted in two deaths. All critical cases occurred in group A (unvaccinated). This group also had all cases of multiple organ dysfunction (14), septic shock (12) and Severe Acute Respiratory Syndrome (15).

In addition, the study found that IgM and IgG antibody levels found in vaccinated patients were significantly higher than in those not immunized. "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," the researchers conclude.

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The roles of inactivated vaccines in older patients with infection of Delta variant in Nanjing, China

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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 electrocardiography, 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 \pm 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 (<i>n</i> , %)	
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 (<i>n</i> , %)	0 (0%)
Septic shock (<i>n</i> , %)	12 (6.7%)
Need for NIV/HFNC (<i>n</i> , %)	33 (18.2%)
Need for MV (<i>n</i> , %)	15 (8.3%)
Need for CRRT/ECMO (<i>n</i> , %)	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 (<i>n</i> , %)	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 (<i>n</i> = 113)	Group B (<i>n</i> = 46)	Group C (<i>n</i> = 22)	<i>P</i> 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 pathophysiology 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.

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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.

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5.5. CoronaVac induces immune response in more than 80% of elderly people, Turkish study shows

A study carried out in Turkey with elderly people immunized with CoronaVac showed once again that the vaccine provides high protection in this population, inducing antibody production in 80.9% of individuals. The study was published in the May issue of the journal *Age & Aging* and was led by researchers from the School of Medicine of the University of Ankara, the Turkish capital.

The scientists evaluated 497 elderly patients with an average age of 72 years who were vaccinated with CoronaVac. The seroconversion rate (individuals who produced antibodies) after one to two months of the second dose was 80.9%, remaining high until the third month, at 73.2%.

The study also pointed out that the presence of comorbidities, such as chronic diseases, can influence the immune response, even more so in older individuals who already have a naturally compromised immune system due to aging. Therefore, it is very important that this population receives the booster dose. The third dose of CoronaVac has already been shown to be effective in boosting the

immunity of the elderly, increasing the neutralizing capacity against SARS-CoV-2 by almost ten times.

Scientific evidence

Several studies carried out with real-world data have already proven that the Butantan and Sinovac vaccine is safe and protects the elderly against severe cases and death. In Project S, for example, a study on the effectiveness of CoronaVac conducted in Serrana, in the São Paulo countryside, the immunizing agent administered to the elderly protected 86.4% against symptomatic cases, 96.9% against hospitalizations and 96.9% against death.

Another Brazilian study carried out with 60 million people, based on national data from the Influenza Epidemiological Surveillance Information System (SIVEP-Gripe) of the Ministry of Health, showed CoronaVac's 84.2% protection against hospitalizations, 80.8% against ICU admissions and 76.5% against death.

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RESEARCH PAPER

Antibody response with SARS-CoV-2 inactivated vaccine (CoronaVac) in Turkish geriatric population

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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, coronovac, 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 inactivated SARS-CoV-2 vaccination.

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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 ($CFS \leq 4$) 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

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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. ^aP-value < 0.05 for the comparison between Group 1, 2 and 3. ^bP value < 0.05 for the comparison between Group 1 and 2. ^cP value < 0.05 for the comparison between Group 1 and 3. ^dP 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

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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

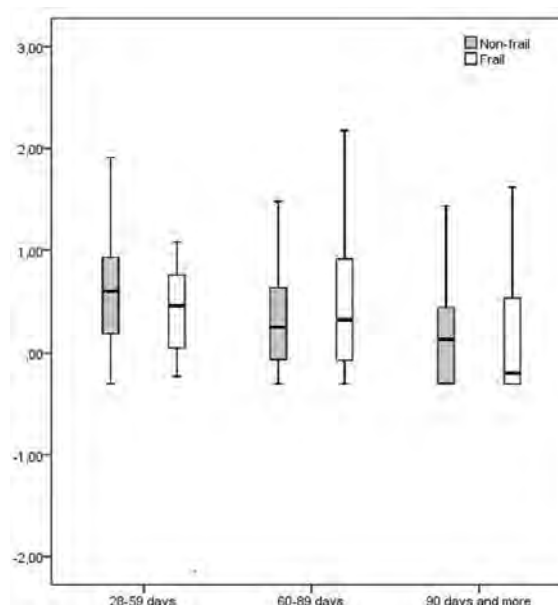


Figure 1. Distribution of antibody titres according to frailty status.

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 drawing 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

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Table 2. Comparison of seropositive and seronegative individuals in Group 1

	Sars-CoV2 spike IgG antibody positive group <i>n</i> = 152 (80.9%)	Sars-CoV2 spike IgG antibody negative group <i>n</i> = 36 (19.1%)	<i>P</i> value
Age, median (IQR)	71 (67–76)	72 (68–80.75)	0.272
Female gender <i>n</i> (%)	87 (58%)	22 (61.1%)	0.734
Comorbidities			
Depression, <i>n</i> (%)	16 (10.2%)	2 (5.6%)	0.533
CVD, <i>n</i> (%)	40 (26.7%)	9 (25.0%)	0.838
HT, <i>n</i> (%)	107 (71.3%)	28 (77.8%)	0.436
DM, <i>n</i> (%)	65 (43.3%)	19 (52.8%)	0.307
Atrial fibrillation, <i>n</i> (%)	13 (8.7%)	3 (8.3%)	0.949
Hypothyroidism, <i>n</i> (%)	27 (18.0%)	7 (19.4%)	0.840
Congestive heart failure, <i>n</i> (%)	13 (8.7%)	6 (16.7%)	0.215
Rheumatological diseases, <i>n</i> (%)	13 (8.7%)	7 (19.4%)	0.074
Malignancy history, <i>n</i> (%)	18 (12.0%)	7 (19.4%)	0.276
Chronic renal disease, <i>n</i> (%)	6 (4.0%)	2 (5.6%)	0.653
Pulmonary diseases, <i>n</i> (%)	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, <i>n</i> (%)	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, <i>n</i> (%)	88 (59.9%)	22 (61.1%)	0.891
Falls, <i>n</i> (%)	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, <i>n</i> (%)	45 (35.7%)	15 (51.7%)	0.111
Low gait speed, <i>n</i> (%)	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 <i>n</i> = 101 (73.2%)	Sars-CoV2 spike IgG antibody negative group <i>n</i> = 37 (26.7%)	<i>P</i> 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, <i>n</i> (%)	15 (15.3%)	4 (11.1%)	0.537
CVD, <i>n</i> (%)	20 (20.4%)	10 (27.8%)	0.364
HT, <i>n</i> (%)	76 (77.6%)	25 (69.4%)	0.334
DM, <i>n</i> (%)	38 (38.8%)	15 (41.7%)	0.742
Atrial fibrillation, <i>n</i> (%)	6 (6.1%)	5 (13.9%)	0.165
Hypothyroidism, <i>n</i> (%)	24 (24.5%)	4 (11.1%)	0.091
Congestive heart failure, <i>n</i> (%)	7 (7.1%)	3 (8.3%)	0.728
Rheumatological diseases, <i>n</i> (%)	2 (2.0%)	7 (19.4%)	0.001*,**
Malignancy history, <i>n</i> (%)	7 (7.1%)	7 (19.4%)	0.055
Chronic renal disease, <i>n</i> (%)	6 (6.1%)	8 (3.8%)	0.701
Pulmonary diseases, <i>n</i> (%)	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, <i>n</i> (%)	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, <i>n</i> (%)	20 (55.6%)	55 (56.7%)	0.816
Falls, <i>n</i> (%)	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, <i>n</i> (%)	25 (30.9%)	7 (23.3%)	0.437
Low gait speed, <i>n</i> (%)	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

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Table 4. Comparison of seropositive and seronegative individuals, in Group 3

	Sars-CoV2 spike IgG antibody positive group <i>n</i> = 98 (57.3%)	Sars-CoV2 spike IgG antibody negative group <i>n</i> = 73 (42.7%)	<i>P</i> value
Age, median (IQR)	75 (68.7–79)	75 (68–81)	0.744
Female gender	63 (64.3%)	4 (5.4.8%)	0.208
Comorbidities			
Depression, <i>n</i> (%)	16 (16.3%)	8 (11%)	0.320
CVD, <i>n</i> (%)	29 (29.6%)	19 (26%)	0.608
HT, <i>n</i> (%)	70 (71.4%)	52 (71.2%)	0.978
DM, <i>n</i> (%)	34 (34.7%)	33 (45.2%)	0.164
Atrial fibrillation, <i>n</i> (%)	15 (15.3%)	10 (13.7%)	0.7691
Hypothyroidism, <i>n</i> (%)	8 (18.4%)	10 (13.7%)	0.414
Congestive heart failure, <i>n</i> (%)	11 (11.3%)	3 (4.1%)	0.090
Rheumatological diseases, <i>n</i> (%)	7 (7.1%)	8 (11%)	0.383
Malignancy history, <i>n</i> (%)	14 (14.3%)	3 (4.1%)	0.052
Chronic renal disease, <i>n</i> (%)	2 (2%)	6 (8.2%)	0.074
Pulmonary diseases, <i>n</i> (%)	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, <i>n</i> (%)	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, <i>n</i> (%)	54 (55.1%)	46 (63%)	0.299
Falls, <i>n</i> (%)	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, <i>n</i> (%)	26 (33.3%)	21 (42%)	0.321
Low gait speed, <i>n</i> (%)	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.

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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-CoV2 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.

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5.6. CoronaVac shows over 70% effectiveness in the elderly in Colombia, study says

A real-world study conducted in Colombia showed over 70% effectiveness of CoronaVac in preventing Covid-19 in the elderly in the country. According to the study, less than 1% of those over the age of 60 immunized with the Butantan and Sinovac vaccine died of the disease. Published in *The Lancet Healthy Longevity*, the research was conducted by the Colombian Ministry of Health and Social Protection and the Universities of the North and Antioquia.

The researchers analyzed data from 2,828,294 people over the age of 60 between March and October 2021, half of whom had been vaccinated against SARS-CoV-2 and the other had not. Since CoronaVac was the first vaccine approved for this population, it was also the most applied immunizing agent (683,284 study participants), followed by AstraZeneca (265,730), Pfizer (400,136) and Janssen (64,997).

CoronaVac results showed an effectiveness of 72.1% against death

in the population over 60 years of age. Less than 1% of the elderly vaccinated with the Butantan immunizing agent died or needed to be hospitalized due to Covid-19. The overall effectiveness of all vaccines in the elderly was 61.6% against hospitalizations and 79.8% against death.

In all the immunizing agents studied, the effectiveness to prevent death was 22.6% lower in older adults over 80 years of age. This is not because vaccines don't work, but because aging itself reduces immune functions naturally.

“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,” the authors point out.

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Effectiveness of COVID-19 vaccines in older adults in Colombia: a retrospective, population-based study of the ESPERANZA cohort



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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.

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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,

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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 titers, 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 7107914 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) RUA-F-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.

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

For the directed acyclic graph see <http://dagitty.net/dags.html?id=W1Li3g>

See Online for appendix

	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 1564092 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 $p < 0\cdot0001$; 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, $p < 0\cdot0001$) across all age groups, with the highest effectiveness in adults aged 60–69 years (76·1% ([71·2–80·2], $p < 0\cdot0001$) and the lowest in adults aged 80 years and older (46·9% [38·5–54·1], $p < 0\cdot0001$).

The effectiveness across all vaccines analysed for preventing death after hospitalisation was 79·8% (95% CI 78·5–81·1, $p < 0\cdot0001$) across all ages, with the highest effectiveness in adults aged 60–69 years (91·0% [89·0–92·6], $p < 0\cdot0001$) and the lowest effectiveness in adults aged 80 years and older (68·4% [65·7–70·9],

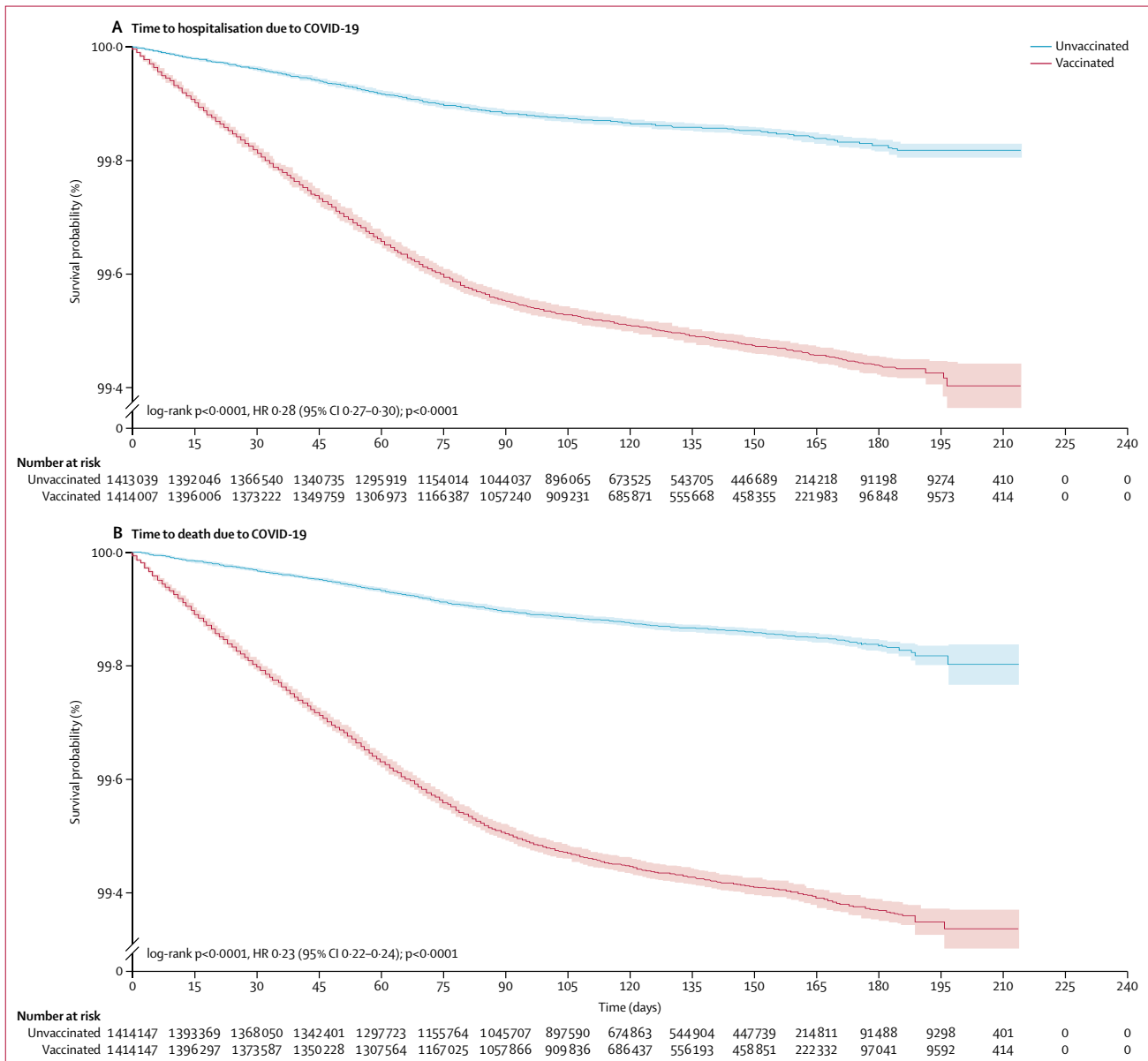


Figure 1: Kaplan-Meier survival curves for adults aged 60 years and older in Colombia (A) Time to hospitalisation due to COVID-19. (B) Time to death due to COVID-19. HR=hazard ratio.

$p < 0.0001$). 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% (95% CI 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).

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

	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

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 study¹³ 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

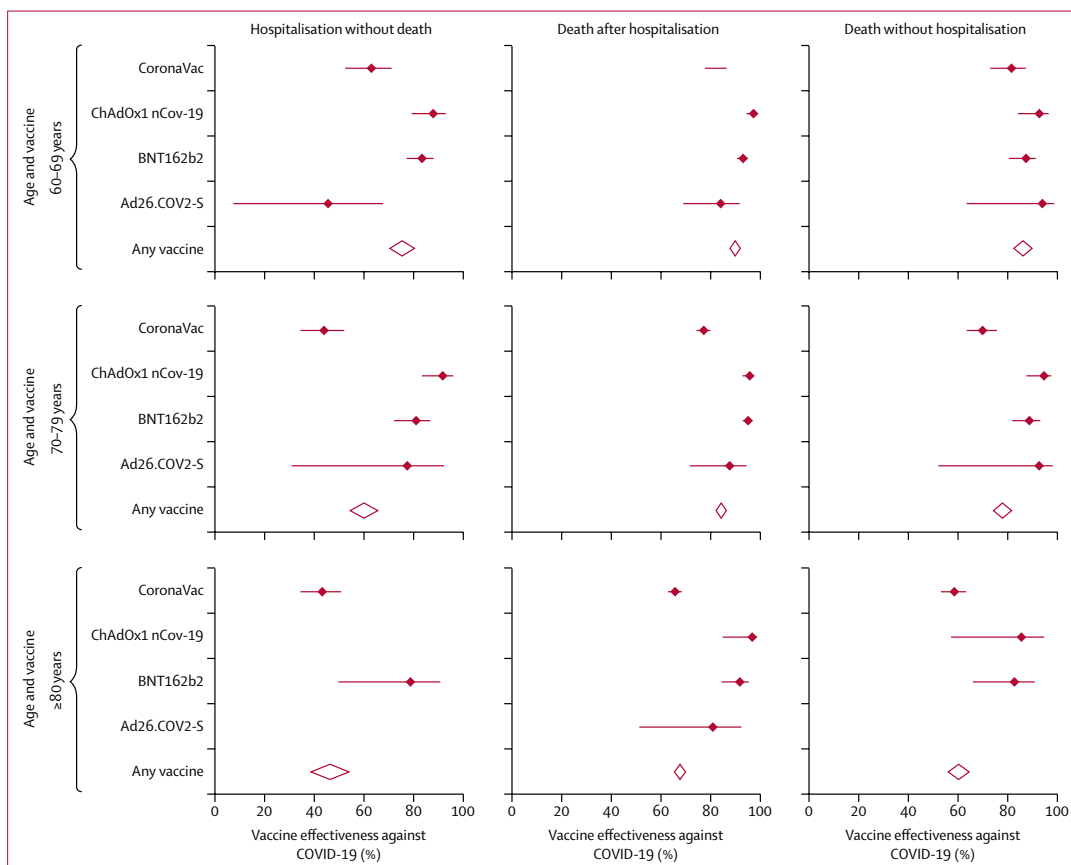


Figure 2: Forest plot of vaccine effectiveness at preventing hospitalisation and death due to COVID-19 in adults aged 60 years and older in Colombia

retrospective cohort study¹⁴ in Catalonia, which analysed 28456 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 study¹⁵ 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 study¹⁷ 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 frailty²⁰ 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 2 066 07 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.COVS-2 and ChAdOx1 nCoV-19, which led to a small number of observed events, especially for Ad26.COVS-2, 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.

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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.

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5.7. Third dose of CoronaVac boosts protection in the elderly to 98% against severe cases and death, study finds

A study conducted by the University of Hong Kong showed that the booster dose of CoronaVac, a vaccine from Butantan and the Chinese pharmaceutical company Sinovac, is 98% effective in protecting elderly people against severe cases and death from Covid-19, even during the outbreak of the SARS-CoV-2 omicron variant. Among those over 80, the third dose protected 96.6% against the same outcome. The study was published on the MedRxiv preprint platform.

The researchers analyzed data on mild and moderate cases (5,474), severe cases (5,294) and deaths (4,093) related to Covid-19, which occurred between December 2021 and March 2022, and investigated the efficacy of two and three doses of CoronaVac and Pfizer, the most used immunizing agents in Hong Kong. With two doses, CoronaVac protected 74% of patients over 60 years of age against severe disease and death, a level similar to that seen with Pfizer. The third dose of the Butantan vaccine boosted protection to 98%.

In younger adults aged 20 to 59, protection with two doses of CoronaVac against severe cases was 91.7% and against death, 94%. With the booster dose, the immunizing agent prevented severe

disease in 98.5% of the individuals (in relation to mortality after the third dose, not enough data were obtained for estimation).

Omicron caused Covid-19 to skyrocket in Hong Kong in January, resulting in 649,454 confirmed cases and nearly 5,000 deaths by mid-March. Vaccination coverage, however, is still below ideal, especially in the elderly group: 66% of people aged between 70 and 79 years and only 37% over 80 years old received both doses. The levels seen for the third dose are even lower, at 30% and 10%, respectively.

“The third dose provides substantial additional protection against severe COVID-19 and should be prioritized, particularly in older adults,” the authors state. They add that Hong Kong is making efforts to expand vaccination coverage in this population, extending the opening hours of immunization clinics and sending vaccination teams to nursing homes, housing projects and for people with reduced mobility.

The Hong Kong results reinforce the findings of other effectiveness studies carried out around the world, which prove that CoronaVac ensures the protection of the elderly.

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1 **Vaccine effectiveness of two and three doses of BNT162b2 and CoronaVac against**
2 **COVID-19 in Hong Kong**

3

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17 Running head: COVID-19 vaccine effectiveness in Hong Kong

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20

21 **Abstract**

22 **Background:** Hong Kong maintained extremely low circulation of SARS-CoV-2 until a
23 major community epidemic of Omicron BA.2 starting in January 2022. Both mRNA
24 BNT162b2 (BioNTech/Fosun Pharma) and inactivated CoronaVac (Sinovac) vaccines are
25 widely available, however coverage has remained low in older adults. Vaccine effectiveness in
26 this predominantly infection-naïve population is unknown.

27 **Methods:** We used individual-level case data on mild/moderate, severe/fatal and fatal
28 hospitalized COVID-19 from December 31, 2021 to March 8, 2022, along with census
29 information and coverage data of BNT162b2 and CoronaVac. We used a negative binomial
30 model, adjusting for age and calendar day to estimate vaccine effectiveness of one, two and
31 three dose schedules of both vaccines, and relative effectiveness by number of doses and
32 vaccine type.

33 **Findings:** A total of 12.7 million vaccine doses were administered in Hong Kong's 7.3
34 million population, and we analyzed data from confirmed cases with mild/moderate
35 (N=5,474), severe/fatal (N=5,294) and fatal (N=4,093) COVID-19. Two doses of either
36 vaccine protected against severe disease and death, with higher effectiveness among adults
37 ≥ 60 years with BNT162b2 (VE: 88.2%, 95% confidence interval, CI: 84.4%, 91.1%)
38 compared to CoronaVac (VE: 74.1%, 95% CI: 67.8%, 79.2%). Three doses of either vaccine
39 offered very high levels of protection against severe outcomes (VE: 98.1%, 95% CI: 97.1%,
40 98.8%).

41 **Interpretation:** Third doses of either BNT162b2 or CoronaVac provide substantial
42 additional protection against severe COVID-19 and should be prioritized, particularly in older
43 adults who received CoronaVac primary schedules. Longer follow-up is needed to assess
44 persistence of different vaccine platforms and schedules.

45 **Funding:** COVID-19 Vaccines Evaluation Program, Chinese Center for Disease Control and
46 Prevention

47 **INTRODUCTION**

48 Hong Kong Special Administrative Region of China (Hong Kong; population 7.3 million) has
49 pursued a COVID-19 elimination strategy since January 2020 involving stringent social
50 distancing measures, border entry restrictions, isolation of cases and quarantine of close
51 contacts, and the use of personal protective measures.¹ Consequently, the disease had been
52 largely controlled through December 2021 with four previous epidemic waves resulting in a
53 total of 12,606 cases (<2 per 1,000) and 207 deaths (<3 per 100,000). Since February 2021,
54 both inactivated (Sinovac; CoronaVac) and mRNA (BioNTech/Fosun Pharma; BNT162b2)
55 vaccines have been widely available with residents offered the choice of either. However, by
56 January 2022, two-dose vaccine coverage had only reached 46% in older adults 70-79 years of
57 age and 18% in those aged ≥ 80 years.²

58

59 A major community epidemic of COVID-19 Omicron variant (B.1.1.529) lineage BA.2 began
60 in early January 2022, resulting in 649,454 laboratory confirmed cases, 313,127 cases reported
61 by rapid antigen tests and nearly 5,000 deaths to March 17, 2022.^{2,3} Vaccination coverage has
62 since risen steadily but remains low in the most vulnerable, with two-dose coverage at 66% and
63 37% in 70-79 and ≥ 80 year olds respectively as of March 17, 2022. Third vaccine doses were
64 recommended first for priority groups and then for the general public on 1 January 2022, to be
65 given six months after the second dose.^{4,5} Third-dose uptake has been highest in the 40-59y age
66 group (46% as of March 17, 2022) and lower in older adults (30% in 70-79 year olds; 10% in
67 those ≥ 80). Efforts to increase vaccine uptake in older and high-risk groups are underway,
68 including reducing the duration between first and second doses for care home residents,
69 extending vaccination clinic operating hours and deployment of vaccine outreach teams to care
70 homes, housing estates and to residents with limited mobility.^{6,7}

71

72 International data has shown vaccination with BNT162b2 reduces the frequency of severe
73 outcomes, and to a lesser extent, infection for variants circulating prior to Omicron.⁸⁻¹⁵ Waning
74 of protection has been observed in multiple contexts, in particular against infection,¹⁶⁻¹⁸ and
75 recent studies have provided early indications of reduced effectiveness of BNT162b2 against
76 the Omicron variant.¹⁹⁻²¹ Evidence on vaccine performance against the more transmissible
77 Omicron subvariant BA.2 remains very limited, as is data on the performance of the inactivated
78 CoronaVac vaccine.²² Limited observational evidence suggests strong and durable protection
79 against severe disease and death, with transient protection against milder symptomatic
80 disease.²³⁻²⁶ With a largely infection-naïve population and two COVID-19 vaccines in
81 widespread use, Hong Kong represents a unique environment for monitoring vaccine
82 effectiveness (VE) against Omicron BA.2. In this study we estimated VE of one, two and
83 three doses of BNT162b2 and CoronaVac, their relative effectiveness, and the additional
84 protection offered by third doses against mild/moderate infections, severe/fatal disease and
85 death.

86

87 **METHODS**

88 *Study design and population*

89 We assessed VE of the BNT162b2 and CoronaVac vaccines using an ecological study design,
90 which has been previously employed to provide estimates of VE in Israel.²⁷ The study
91 population consisted of residents of Hong Kong aged 20 years and over, where the population
92 with zero, one, two or three doses of either vaccine at risk at a given time was derived using
93 detailed data from the vaccination programme and population census. Information on all
94 laboratory-confirmed SARS-CoV-2 cases in Hong Kong from December 31, 2021 to March
95 8, 2022 was obtained from nationwide individual level surveillance data provided by the

96 Centre for Health Protection and linked to clinical outcome data provided by the Hospital
97 Authority.

98

99 *Ethical approval*

100 This project received approval from the Institutional Review Board of the University of Hong
101 Kong.

102

103 *Infections and outcomes*

104 Extensive PCR testing for SARS-CoV-2 is conducted in public hospitals, community test
105 centres and private laboratories in Hong Kong. Testing is free-of-charge or available at low
106 cost, and required for those who exhibit COVID-19 like symptoms, or following contact
107 tracing based on exposure history or residential location. Regular screening is also required of
108 certain professions, in particular those working with older adults or vulnerable persons.
109 Positive rapid test results have been recognised as confirmed infections since February 25,
110 2022 and included in official case counts from March 7, 2022. Data on all laboratory-
111 confirmed cases between December 31, 2021 and March 8, 2022 were extracted and cases
112 classified as ‘imported’, i.e. detected in on-arrival quarantine, were excluded due to their non-
113 representative SARS-CoV-2 exposure and vaccination histories. Sequencing of a subset of
114 cases each day indicates that fewer than 1% of cases and deaths during the fifth wave have
115 occurred with the Delta variant, with the remaining infections attributed to the Omicron BA.2
116 lineage.

117

118 Hong Kong has an advanced public and private healthcare system whereby private clinics
119 comprise most primary care and government hospitals provide approximately 90% of hospital
120 medical services at very low cost to patients.²⁸ Up until mid-February 2022, all laboratory-

121 confirmed COVID-19 cases were admitted to hospitals for isolation and standardized clinical
122 management, regardless of symptom presentation, with their hospitalization records stored in
123 the data system managed by the Hospital Authority. After mid-February 2022, due to the
124 large number of incident cases, hospitalisation was reserved for patients with more severe
125 disease, and milder cases were required to isolate at dedicated government quarantine
126 facilities or at home. In the Hospital Authority data system, records of patients' test results,
127 medication and condition changes were documented and integrated into a centralized
128 database from which we extracted relevant information on those experiencing mild/moderate
129 disease prior to February 16, 2022 and severe disease and death at any time. We excluded
130 those with conflicting information in the database, i.e. persons with a worst recorded
131 condition of 'mild' but also experiencing a fatal outcome within hospital. Severe disease was
132 defined as any severe, critical or fatal COVID-19 case (definitions for each in Appendix).

133

134 *Population uptake of COVID-19 vaccines*

135 Data on the estimated population size at the end of 2021 by age and sex were obtained from the
136 Census and Statistics Department of the Hong Kong Special Administrative Region
137 Government. Data on the number of persons vaccinated with either the BNT162b2 or
138 CoronaVac vaccines in Hong Kong each day since February 22, 2021 are available in a
139 national vaccination database provided by the Department for Health. Data on all vaccinations
140 that had occurred up to March 8, 2022, including vaccinee age and the type and date of receipt
141 of each dose of vaccine, were extracted on March 10, 2022. Vaccination information for all
142 cases in the surveillance data was cross checked with Hospital Authority records and any cases
143 with discrepancies were excluded.

144

145 Those who received vaccines other than BNT162b2 or CoronaVac, or who received a mixed
146 primary series of one dose of BNT162b2 and one dose of CoronaVac, were excluded from the
147 analysis. In addition, for the purposes of this analysis we also exclude those who switched
148 vaccine platform after the second dose, that is, those who received two doses of CoronaVac and
149 a third dose of BNT162b2 and those who have received a primary series of BNT162b2 and a
150 third dose of CoronaVac. Cases with known prior COVID-19 infection were also excluded.

151

152 *Statistical analysis*

153 Incidence rates were calculated according to the number of doses of COVID-19 vaccination
154 received (none, one, two or three) for each age group (20-29, 30-39, 40-49, 50-59, 60-69, 70-
155 79, ≥ 80 years) and calendar day throughout the study period. Additional stratification by
156 vaccine type was included to estimate VE for each vaccine type and relative VE (rVE)
157 between two and three doses of each vaccine. Vaccination status was categorised according to
158 the date of vaccination plus a 14-day lag for all doses, to allow for the delay in immune
159 response to vaccination. Daily numbers of persons in each vaccination category were inferred
160 from the uptake data assuming that individuals received the same vaccine for first and second
161 dose (aligned with Hong Kong guidelines), and using aggregate data by age on vaccine
162 switching for the third dose. The population at risk in each stratum was matched to the report
163 date of cases, and cumulative numbers of previous SARS-CoV-2 infections within each
164 group were removed from the population at risk at each time point. Incidence rate ratios
165 (IRR) were estimated using a negative binomial rate model for the daily counts of cases
166 adjusted for age group and calendar day including the logarithm of person-time as an offset
167 term in the model to account for differing numbers at risk within each strata. VE was defined
168 as $(1-IRR)\times 100\%$.

169

170 **RESULTS**

171 A total of 486,074 persons had confirmed SARS-CoV-2 infection during the study period
172 from December 31, 2021 to March 8, 2022. The case data were linked to the Hospital
173 Authority dataset to determine their clinical outcomes and those with complete age and
174 vaccination records were extracted. Of these, 5,474 persons were recorded as having
175 mild/moderate disease between December 31, 2021 and February 15, 2022. During the entire
176 study period from December 31, 2021 to March 8, 2022, 5,294 persons with severe/fatal
177 disease and 4,093 with fatal disease were included (Table 1).

178

179 Up to March 8, 2022, a total of 12.7 million vaccine doses had been administered in Hong
180 Kong. Severe disease or death occurred a median of 161 (interquartile range, IQR: 73 to 207)
181 days after the second vaccination in those vaccinated with two doses of BNT162b2, and 127
182 (IQR: 51 to 162) among those who received two doses of CoronaVac. Those experiencing
183 severe and fatal outcomes after a third dose tested positive a median of 52 (IQR: 38 to 70)
184 days and 45 (IQR: 24 to 100) days after vaccination with BNT162b2 and CoronaVac
185 respectively. The distribution of mild cases according to age and vaccination status were
186 similar to the population, with severe disease and death occurring predominantly in the
187 unvaccinated older population (Figure 2).

188

189 ***VE after receipt of two doses***

190 We found two doses of CoronaVac provided no protection against mild/moderate disease
191 across all age groups, with some protection offered by BNT162b2 in younger age groups
192 (VE: 31.0%, 95% CI: 1.6%, 51.7%). However, both vaccines were estimated to have high
193 effectiveness against severe disease. Limited differences in vaccine effectiveness were
194 observed for severe outcomes in younger adults, where VE was estimated to be 95.2% (95%

195 CI: 92.9%, 96.8%) for BNT162b2 and 91.7% (95% CI: 87.8%, 94.4%) for CoronaVac (Table
196 2). The difference in VE was more pronounced for older adults, with higher effectiveness
197 among adults >60 years who received BNT162b2 (VE: 88.2%, 95% confidence interval, CI:
198 84.4%, 91.1%) compared to CoronaVac (VE: 74.1%, 95% CI: 67.8%, 79.2%). When broken
199 down further by age, we estimated that VE was 91.1% (95% CI: 85.4%, 94.6%) for
200 BNT162b2 and 82.6% (74.2%, 88.2%) for CoronaVac in those 60-69y, reducing to 84.5%
201 (95% CI: 75.5%, 90.2%) and 60.2% (95% CI: 43.9%, 71.8%) among those ≥ 80 y for
202 BNT162b2 and CoronaVac, respectively. This was also observed for the mortality endpoint,
203 where in adults aged ≥ 80 y two doses of BNT162b2 offered a higher level of protection
204 against fatal disease (88.2%, 95% CI: 80.2%, 93.0%) compared to two doses of CoronaVac
205 (66.8%, 95% CI: 51.9%, 77.0%).

206

207 We compared the two-dose schedules of both vaccines and found no significant differences
208 between BNT162b2 and CoronaVac for mild disease in any age group. Superiority of the
209 two-dose BNT162b2 schedule was estimated for severe/fatal disease in adults ≥ 60 y (relative
210 VE: 54.6%, 95% CI: 38.7%, 66.4%). This was also the case for mortality in those ≥ 60 y
211 (relative VE: 58.5%, 95% CI: 70.7%, 41.3%). No differences between vaccines were found
212 against severe/fatal or fatal COVID-19 in adults 20-59y.

213

214 *VE after receipt of three doses*

215 We estimated three doses of both vaccines offered very high protection against severe disease
216 (98.1%, 95% CI: 97.1%, 98.8%) and mortality (98.6%, 95% CI: 97.7%, 99.2%) which was
217 sustained within all age groups (Table 2). Vaccine estimates were very similar for both
218 vaccines against severe and fatal outcomes. Three doses of BNT162b2 was estimated to have
219 a VE of 71.5% (95% CI: 54.5%, 82.1%) against mild/moderate disease in younger adults

220 while for three doses of CoronaVac the VE was estimated as 42.3% (95% CI: 11.4%, 62.4%)
221 against the same outcome.

222

223 *Relative VE of three versus two doses*

224 We estimated the relative effect of three doses versus two doses of each vaccine type (Table
225 3). For mild/moderate disease we find an additional benefit of a third dose of BNT162b2 in
226 younger (relative VE: 58.6%, 95% CI: 34.4%, 73.9%) and older (relative VE: 63.8%, 95%
227 CI: 26.7%, 82.1%) adults who had previously received two doses of BNT162b2. A third dose
228 of CoronaVac increased protection (relative VE: 57.0%, 95% CI: 23.4%, 75.9%) in older
229 adults who had received two doses of CoronaVac, with no benefit observed in the younger
230 age category. For severe/fatal disease we found an additional benefit of a third dose in adults
231 of all ages for both vaccine types, with relative VE of 71.9% (95% CI: 25.1%, 89.5%) for
232 three vs two doses of BNT162b2, and 96.6% (95% CI: 85.7%, 99.2%) for three vs two doses
233 of CoronaVac among those ≥ 80 years. Additional protection against mortality was offered by
234 a third dose in older adults, with no differences observed in younger adults.

235

236 **DISCUSSION**

237 We used detailed population-level data on the vaccination programme in Hong Kong since
238 February 2021 and individual-level COVID-19 case data from December 31, 2021 to March
239 8, 2022 to estimate VE of one, two and three doses of BNT162b2 and CoronaVac vaccines in
240 a largely infection-naïve population during the fifth wave of COVID-19 in Hong Kong. Two
241 or three doses of BNT162b2 or three doses of CoronaVac provide a very high level of
242 protection against severe disease and death in those under 80 years of age. A reduction in VE
243 was observed among two-dose CoronaVac recipients ≥ 80 years. We found no effect of two
244 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

270 VE: 90.9%, 95% CI: 78.6%, 96.1%).³⁵ Similarly, a US study estimated VE of two doses of
271 mRNA vaccines against severe Omicron disease, defined as COVID-19 requiring invasive
272 mechanical ventilation or in-hospital death, of 79% (95% CI: 66%, 87%) a median of 265
273 days after the second dose; and three dose VE of 94% (95% CI: 88%, 97%), similar to our
274 estimate of 98.1% (95% CI: 97.1%, 98.8%).³⁶

275

276 Despite the overall consistency between our results and those presented in other studies, it is
277 possible that VE, particularly against severe outcomes, has been overestimated in our study.
278 Vaccine hesitancy in Hong Kong is highest among the elderly and in individuals with
279 underlying health conditions.³⁷ In this scenario so-called ‘healthy vaccinee bias’, by which
280 vaccine recipients are healthier than their unvaccinated peers, may inflate the estimates.³⁸
281 Although we have accounted for age in the current estimates, a lack of individual-level data
282 on controls mean that this cannot be formally assessed with currently available data.
283 However, our estimates for BNT162b2 and CoronaVac are similar to other studies using
284 alternative designs, and we anticipate the magnitude of overestimation is unlikely to be
285 substantial.^{19,35} Even if individual-level adjustments had been possible, estimating absolute
286 VE after vaccines have been available for some time is problematic because it is necessary to
287 compare incidence rates in vaccinated individuals with those from unvaccinated cohorts often
288 with few remaining persons. This is the case in younger age groups in Hong Kong, whose
289 characteristics are likely to differ substantially from those who chose to be vaccinated earlier.
290 This bias, inherent to observational studies, is present in much of the existing VE literature at
291 this stage of the pandemic. To address this concern, we also estimated a relative VE of three
292 versus two doses of each vaccine type, as these cohorts are likely to be more comparable
293 (Table 3). We find a third dose of either vaccine provides additional protection, reiterating the
294 public health value of a third dose for minimizing severe disease and death but also for

295 reducing health system congestion, public concern and indirect costs stemming from milder
296 episodes during a COVID-19 epidemic.

297

298 We compared performance of the mRNA BNT162b2 and inactivated CoronaVac vaccines
299 and found higher VE for BNT162b2 following one and two doses, but similar performance
300 after three doses (Table 2). Our estimates are likely to be affected by time since vaccination,
301 where typically more time has passed since administration of second than third doses which
302 have only been widely available in Hong Kong since the beginning of January 2022 (Table
303 1). Improved effectiveness may partially reflect a recent, rather than a third, vaccine dose.
304 This hypothesis is supported by data from an observational study in Malaysia which
305 compared the duration of protection of the BNT162b2 and CoronaVac vaccines. They find
306 more rapid waning of CoronaVac, in particular for mild/moderate and severe outcomes, but
307 to a lesser extent for COVID-19 related mortality.²⁴ Moreover, a recent study of humoral and
308 cellular responses among Hong Kong vaccinees over time found that neutralising antibodies
309 against variants of concern dropped to detection limit only three months after vaccinations,
310 along with diminishing memory T cell responses, primarily among CoronaVac recipients.³⁹

311

312 Our study has a number of limitations arising from available data and the nature of the
313 epidemic within Hong Kong. Firstly, we used census data from the correct time period to
314 construct the source population, but any differential population movement by vaccine status
315 over the duration of the vaccination program could affect the validity of our estimates.
316 Furthermore, as we are estimating vaccine effectiveness in real-time, there are large amounts
317 of missingness in clinical data, which is especially problematic when assuming a population
318 level denominator, as the assumed number of people still at risk will be overestimated.
319 However, this is mostly an issue for mild/moderate outcomes, as we used complete records

320 on COVID-19 mortality to derive estimates and we expect severe cases are fully documented.
321 Secondly, there are some differences in testing requirements by vaccine status, particularly
322 for those required to regularly test because of occupation. However, we expect that VE
323 estimates against severe outcomes will be only marginally susceptible to biases related to
324 testing requirements. Finally, in Hong Kong there was a clear preference for the BNT162b2
325 vaccine in younger age groups and for CoronaVac in older adults. We have addressed this
326 confounding in estimates presented by stratifying by age categories and adjusting estimates
327 by 10-year age categories and calendar day, however some residual confounding by age is
328 possible in the vaccine platform-specific estimates and other factors may confound the
329 relationship between vaccine status, type and risk of infection that cannot be accounted for in
330 this design.

331

332 Our findings indicate that two dose schedules of both BNT162b2 and CoronaVac vaccines
333 offer strong protection against severe disease and death, however higher levels of protection
334 were observed among those who received two doses of BNT162b2 compared to those
335 receiving two doses of CoronaVac, particularly in older age groups. Three recent doses of
336 both vaccines offer very high levels of protection for older adults against severe outcomes,
337 with no differences observed across vaccine types. It will be important to increase uptake of
338 third vaccine doses, particularly in older adults who have so far received two doses of
339 CoronaVac. Further investigation of the durability of protection provided by both vaccines is
340 warranted and planned.

341

342

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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

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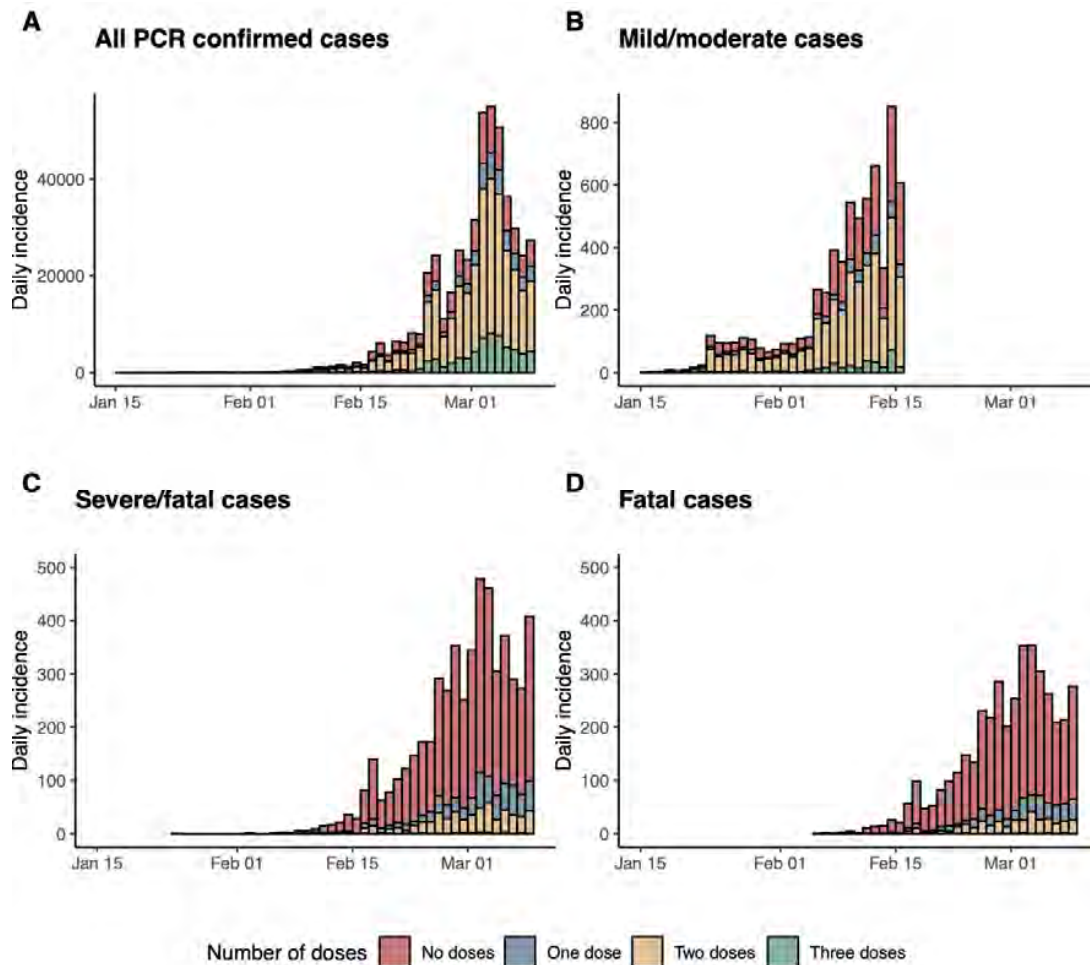
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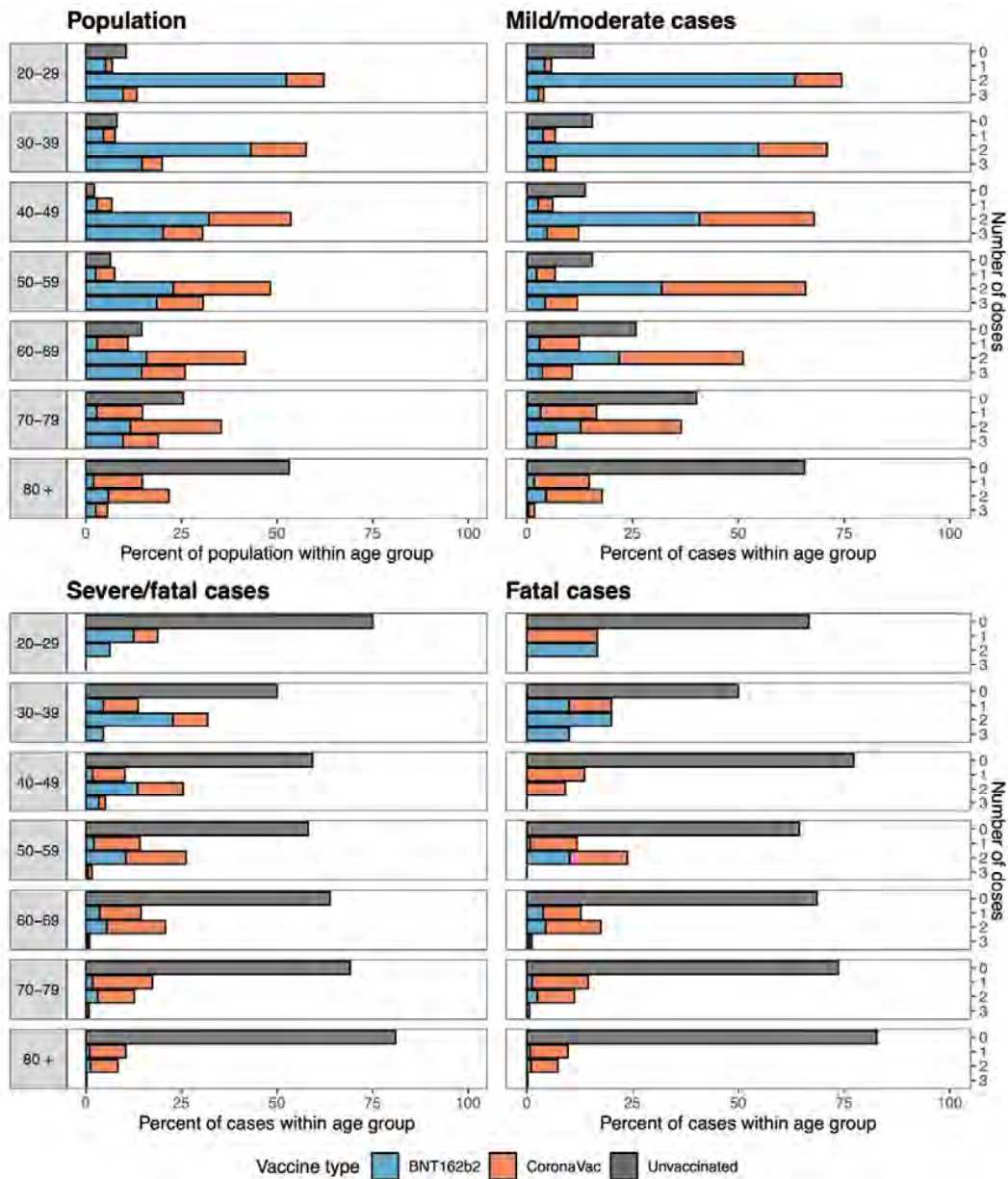
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496

497 Figure 1. Daily incidence of (A) all PCR confirmed COVID-19 cases (B) mild/moderate
 498 cases in the early part of the fifth wave prior to 15 February 2022, (C) severe/fatal cases, and
 499 (D) deaths throughout the fifth wave in Hong Kong by vaccination status, where severe
 500 disease is defined as having ever been listed as ‘Serious’ or ‘Critical’ or ‘Fatal’ by the
 501 Hospital Authority during hospitalisation for COVID-19. Vaccination status was categorised
 502 according to the number of doses received plus a 14-day lag for all doses, to allow for the
 503 immune response to vaccination. The drop in mild/moderate cases on 4 March was due to a
 504 very small number of cases being reported as having been admitted to hospital or isolation
 505 facilities on that day. Mild cases were only included up until 15 February 2022 to account for
 506 change in admission criteria.

507



508

509 Figure 2. Vaccine status of population and those experiencing mild/moderate, severe/fatal
 510 and fatal COVID-19 as at 8 March 2022 as a percent of the population within a given age
 511 group shown by vaccine type and number of doses.

512

513

516 Table 1. Descriptive characteristics of confirmed COVID-19 cases in Hong Kong classified
517 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			
<i>BNT162b2</i>	151	73	44
<i>CoronaVac</i>	226	532	374
Two doses			
<i>BNT162b2</i>	2139	130	74
<i>CoronaVac</i>	1271	434	287
Three doses			
<i>BNT162b2</i>	126	12	7
<i>CoronaVac</i>	210	14	7
Median (25th, 75th percentile) of days between last vaccine dose and positive SARS-CoV-2 test result^b			
One dose			
<i>BNT162b2</i>	27 (22, 35)	21 (18, 32)	21 (18, 32)
<i>CoronaVac</i>	29 (21, 35)	24 (17, 38)	24 (17, 39)
Two doses			
<i>BNT162b2</i>	182 (151, 217)	161 (73, 207)	171 (91, 213)
<i>CoronaVac</i>	179 (146, 209)	127 (51, 162)	125 (51, 157)
Three doses			
<i>BNT162b2</i>	31 (20, 49)	52 (38, 70)	68 (49, 77)
<i>CoronaVac</i>	39 (25, 66)	45 (24, 100)	64 (30, 100)

518 ^aNumber of doses plus 14-day lag

519 ^bMedian time since vaccination among those where 14 days has passed since latest dose

520

521

522 Table 2. Vaccine effectiveness by dose (one, two, three) and vaccine type (CoronaVac,
 523 BNT162b2) in all ages and within age categories (mild/moderate: 20-59, ≥ 60 ; severe/fatal,
 524 fatal: 20-59, 60-69, 70-79, ≥ 80 years) against COVID-19 related mild/moderate disease,
 525 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 (0.7, 60.6)	2.1 (-53.3, 37.5)	31.0 (1.6, 51.7)	17.9 (-18.0, 42.9)	71.5 (54.5, 82.1)	42.3 (11.4, 62.4)
≥ 60 years	None ^a	None ^a	None ^a	None ^a	71.6 (43.5, 85.7)	50.7 (12.9, 72.1)
Severe/fatal disease						
20-59 years	85.0 (69.1, 92.7)	60.9 (40.6, 74.3)	95.2 (92.9, 96.8)	91.7 (87.8, 94.4)	98.5 (95.9, 99.4)	98.5 (95.2, 99.5)
60-69 years	59.9 (29.3, 77.3)	55.1 (30.9, 70.9)	91.1 (85.4, 94.6)	82.6 (74.2, 88.2)	99.2 (96.7, 99.8)	98.5 (95.3, 99.6)
70-79 years	71.5 (48.9, 84.1)	33.9 (8.1, 52.5)	89.4 (83.0, 93.3)	80.8 (72.8, 86.5)	99.5 (96.0, 99.9)	96.7 (92.3, 98.6)
≥ 80 years	65.0 (42.2, 78.8)	35.0 (8.8, 53.7)	84.5 (75.5, 90.2)	60.2 (43.9, 71.8)	95.7 (89.0, 98.3)	98.6 (94.3, 99.7)
Mortality						
20-59 years	93.7 (74.2, 98.5)	65.4 (38.6, 79.4)	96.4 (93.6, 98.0)	94.0 (89.6, 96.5)	99.4 (95.6, 99.9)	- ^b -
60-69 years	63.3 (30.7, 80.5)	70.2 (51.3, 81.7)	93.7 (88.6, 96.5)	87.6 (80.9, 91.9)	98.9 (95.3, 99.7)	98.7 (94.4, 99.7)
70-79 years	81.3 (60.6, 91.1)	48.9 (28.1, 63.7)	92.2 (86.5, 95.5)	84.4 (77.5, 89.2)	- ^b -	97.2 (92.3, 99.0)
≥ 80 years	71.8 (50.6, 83.9)	40.5 (14.9, 58.4)	88.2 (80.2, 93.0)	66.8 (51.9, 77.0)	96.0 (88.8, 98.6)	99.2 (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

533 Table 3. Relative vaccine effectiveness of a three versus two dose BNT162b2 schedule and a
 534 three versus two dose CoronaVac schedule against mild disease, severe disease and mortality
 535 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)

536
 537 ^a Insufficient outcomes to estimate

538

5.8. Chilean study with more than 10 million people demonstrates that the effectiveness of CoronaVac is higher than 86%, including among the elderly

An article published in the The New England Journal of Medicine showed once more that CoronaVac, vaccine from Butantan and the chinese pharmaceutical Sinovac, is effective (which means, has proved efficacy in the “real world” and not only in a controlled study of clinical trials) against cases of Covid-19 and variants of the SARS-CoV-2, including among the people with more than 60 years of age.

The research was realized in Chile, and showed that the protection of CoronaVac was 65,9% against Covid-19 infections, 87,5% against hospitalization, 90,3% against hospitalizations in the Intensive Care Unit (ICU) and 86,3% against deaths. For the group fully vaccinated above 60 years of age, the effectiveness of the vaccine was 66,6% for protection against infections, 85,3% against hospitalization, 89,2% against hospitalizations in the Intensive Care Unit (ICU) and 86,5% for prevention of deaths related to the disease.

The analysis was made between February and May of 2021 with around 10,2 million people. The cohort study (observational study that follows individuals during a period of time to determine characteristics and evolution of the group) had participants with more than 16 years old registered in the National Found

of Health (FONASA), chilean national program of health, that covers around 80% of the population.

The participants were divided into three groups: non vaccinated, vaccinated with only one dose and totally vaccinated. The tests for detection of Covid-19 were RT-PCR (98,1%) and quick antigen tests (1,9%). During the period of analysis, the ICUs in Chile operated with 93,5% of its capacity.

The andean country has the highest rates on test realization for Covid-19 detection in Latin America and a padronized system of public information with statistics that were vital for this study. The Health Ministry of Chile used 13,98 million doses of the CoronaVac vaccine since the beginning of the vaccination campaign, in February 2021.

Another effectiveness study of CoronaVac was made by Butantan in Serrana, São Paulo. Project S vaccinated almost the whole adult population of the city (28,000 people) between February and April 2021 and concluded that the immunizer caused a reduction of 80% in the number of symptomatic cases of Covid-19, 86% in hospitalization and 95% in deaths.

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Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile

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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.

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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,^{9,12} 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 Israel¹⁵ and Scotland¹⁶ 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 coun-

tries 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 vaccine²² were carried out in China among participants 18 to 59 years of age²³ 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-

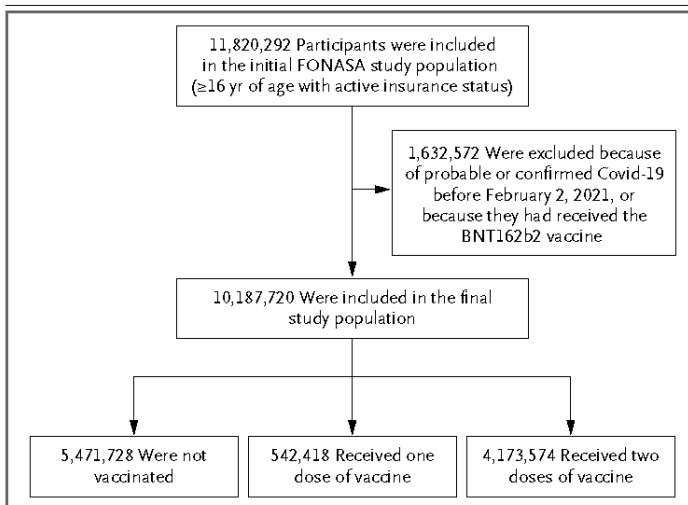


Figure 1. Study Participants and Cohort Eligibility.

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

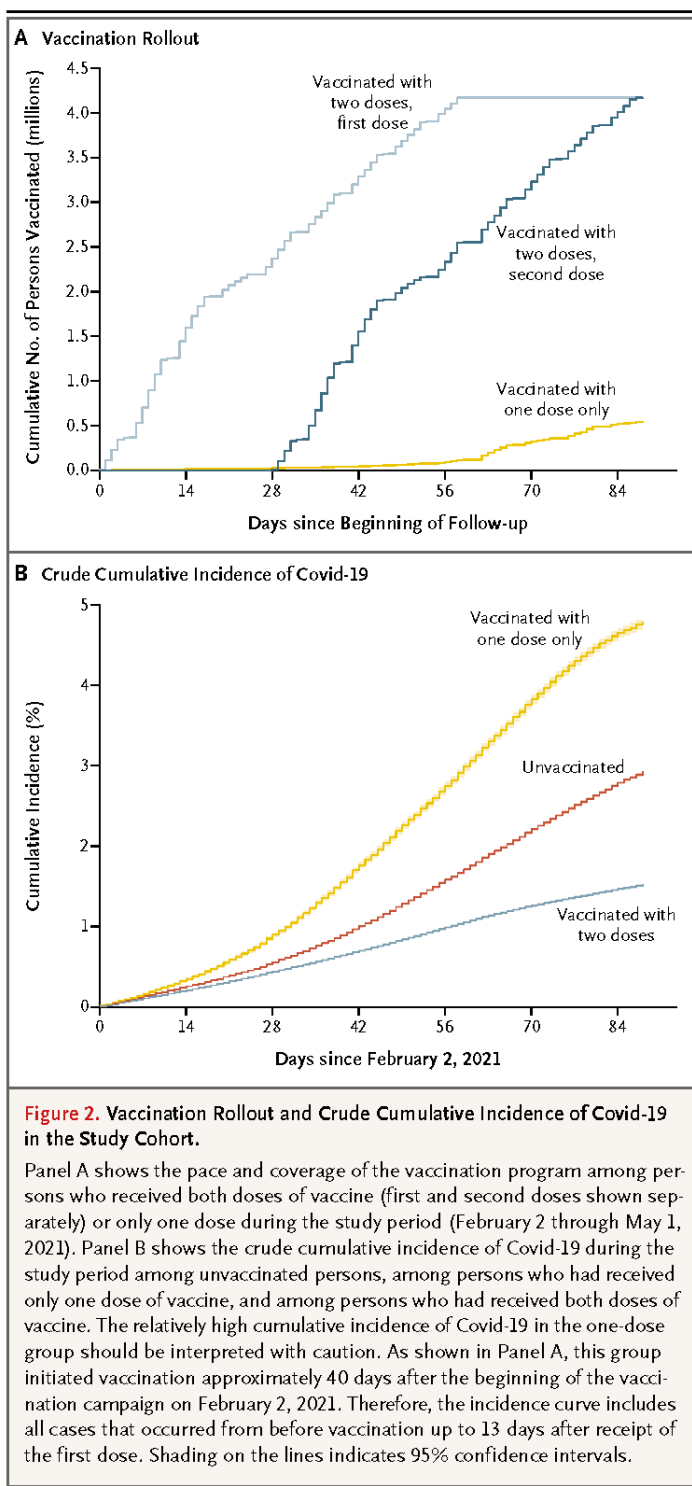
INACTIVATED SARS-COV-2 VACCINE IN CHILE

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	<0.001
No. of coexisting conditions†												
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	14.9	

* 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.



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

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

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 No. of Person-Days	Persons with Covid-19		Vaccine Effectiveness (95% CI)		
		No. of Persons	Incidence Rate <i>no. of events/ 1000 person-days</i>	Analysis Adjusted for Sex and Age	Analysis Adjusted for All Covariates†	Stratified Analysis‡
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 No. of Person-Days	Persons with Covid-19		Vaccine Effectiveness (95% CI)		
		No. of Persons	Incidence Rate <i>no. of events/ 1000 person-days</i>	Analysis Adjusted for Sex and Age	Analysis Adjusted for All Covariates*	Stratified Analysis†
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 effec-

tiveness 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 disease³ 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.³³ 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 con-

sidered 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 Brazil⁴²), 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.

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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>.

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5.9. Study with 60 million Brazilians demonstrates an effectiveness of CoronaVac higher than 70% against hospitalizations and deaths, even among elderlies

A research made with 60,5 million of vaccinated Brazilians between January and June of 2021 demonstrated that CoronaVac, vaccine of Butantan and the Chinese pharmaceutical Sinovac, have an effectiveness higher than 70% to avoid severe cases, hospitalization in the Intensive Care Unit (ICU) and deaths caused by Covid-19, even among the elderlies. The study, which analyzed CoronaVac and the vaccine of AstraZeneca/Fiocruz, is the biggest realized in Brazil about the effectiveness of the vaccination against SARS-CoV-2.

From the total number of evaluated people that had completed the vaccination scheme with CoronaVac (which means, had received both doses), 72,6% presented less risk of hospitalization, 72,4% less risk of admission in an ICU and 74% less risk of death. Among the people between 60 and 89 years of age, the effectiveness of the vaccine was even better: 84,2% against hospitalization, 80,2% against hospitalizations in ICU and 76,5% against deaths.

The study was realized by researchers from the federal universities of Bahia and Ouro Preto, the Brasilia University, the State University of Rio de Janeiro, from London School of Hygiene & Tropical Medicine and Oswaldo Cruz Foundation (Fiocruz). The conclusions were published in the article “The effectiveness of Vaxzevria and CoronaVac vaccines: A nationwide longitudinal retrospective study of 61 million Brazilians (VigiVac-COVID19)”, in the preprint platform MedRxiv.

From the 60,5 million Brazilians analyzed in the study, 21,9 million (36,2%) were immunized with CoronaVac, and 38,6 million (63,8%) with the vaccine of AstraZeneca/Fiocruz. In total, 26,8 million people (44,4% in total) were 60 or older.

To determine the effectiveness of the vaccines in avoiding severe cases of Covid-19, the researchers confronted the informations of vaccinated population with the national data of the Epidemiological Vigilance of Influenza System

(SIVEP-Gripe), that gather notified cases of hospitalization and deaths caused by respiratory viruses, which is the case of SARS-CoV-2.

The study is extremely important not just because of the high number of analyzed individuals, but also for being the first data survey made nationally to measure the vaccinal effectiveness - which is not the same as efficacy. While the investigation of efficacy is made in ideal and controlled conditions, usually in labs, the vaccinal effectiveness analysis is based on data of the real world, where the vaccine is put on proof in a diverse group of people, in different conditions.

Another effectiveness study related to CoronaVac is the Project S, made by Butantan in Serrana, countryside of São Paulo. Through that study, the population (almost 28,000 adult people) were vaccinated between February and April of 2021.

The research concluded that the immunizer caused a reduction of 80% in the number of symptomatic cases of Covid-19, 86% in the hospitalizations and 95% in deaths. Besides, it showed that with a vaccine coverage of about 75% of the adult population the pandemic can be controlled.

The efficacy of CoronaVac was proved in Brazil through a clinical study of phase 3 with 13,000 volunteers, all healthcare workers, a highly exposed population to Covid-19. The final results demonstrated that the general efficacy of the vaccine can reach 62,3% when the gap between the first and second dose is from 21 to 28 days. The data was disclosed on the preprint platform SSRN, associated with The Lancet journal.

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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

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Article

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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 Coronavac. 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 trial⁸ 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 $1 - \text{HR}$, 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 Vaxzevria 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 study²⁴, 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 Scotland³ 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 by 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 controlled 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.

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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/>

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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)

<i>30-39</i>	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)
<i>40-49</i>	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)
<i>50-59</i>	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)
<i>60-69</i>	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)
<i>70-79</i>	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)
<i>80-89</i>	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)
<i>≥90</i>	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						
<i>Central West</i>	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)

<i>Northeast</i>	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)
<i>North</i>	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)
<i>Southeast</i>	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)
<i>South</i>	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)
<i>Missing</i>	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						
<i>I</i>	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)

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)
<i>Missing</i>	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 or 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								
<i>Reference period</i>	474,317,595	76,780	0,1619	Ref	272,340,929	47,523	0,1745	Ref
<i>Partially vaccinated</i>	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)
<i>Fully</i>	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)

<i>vaccinated</i>				71.3)					
Hospitalization									
<i>Reference period</i>	474,679,253	18,420	0.0389	Ref	272,540,206	15,080	0.0553	Ref	
<i>Partially vaccinated</i>	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)	
<i>Fully vaccinated</i>	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									
<i>Reference period</i>	474,760,394	6,272	0.0132	Ref	272,599,778	5,643	0.0207	Ref	
<i>Partially</i>	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)	

<i>vaccinated</i>				55.6)				
<i>Fully vaccinated</i>	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								
<i>Reference period</i>	474,761,099	6,255	0.0131	Ref	272,587,083	7,529	0.0276	Ref
<i>Partially vaccinated</i>	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)
<i>Fully vaccinated</i>	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.

* 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				
<i>Reference period</i>	—	—	—	—
<i>Partially vaccinated</i>	27.4 (26.5-28.2)	34.0 (33.2-34.7)	14.1 (12.9-15.3)	16.4 (15.2-17.5)
<i>Fully vaccinated until 13 days</i>	49.0 (47.3-50.6)	56.9 (55.3-58.5)	38.2 (37.2-39.1)	40.3 (39.4-41.2)
<i>Fully vaccinated</i>	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				
<i>Reference period</i>	—	—	—	—
<i>Partially vaccinated</i>	45.3 (43.8-46.7)	52.2 (50.9-53.4)	24.1 (22.1-26.0)	26.6 (24.6-28.4)
<i>Fully vaccinated until 13 days</i>	53.8 (50.5-56.9)	69.6 (67.2-71.8)	55.0 (53.6-56.4)	57.3 (56.0-58.6)
<i>Fully vaccinated</i>	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				
<i>Reference period</i>	—	—	—	—
<i>Partially vaccinated</i>	46.5 (44.0-48.9)	54.0 (51.8-56.0)	25.3 (22.1-28.4)	28.1 (24.9-31.1)

<i>Fully vaccinated until 13 days</i>	51.5 (45.6-56.8)	69.2 (65.0-72.8)	55.8 (53.5-57.9)	58.1 (55.9-60.1)
<i>Fully vaccinated</i>	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				
<i>Reference period</i>	—	—	—	—
<i>Partially vaccinated</i>	39.7 (37.0-42.3)	49.3 (47.0-51.5)	26.9 (24.2-29.6)	29.4 (26.7-32.0)
<i>Fully vaccinated until 13 days</i>	31.9 (24.9-38.3)	72.1 (69.1-74.9)	56.2 (54.3-58.1)	58.7 (56.9-60.4)
<i>Fully vaccinated</i>	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										
<i>Partially vaccinated</i>	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)
<i>Fully vaccinated until 13 days</i>	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)
<i>Fully vaccinated</i>	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										
<i>Partially vaccinated</i>	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)

<i>Fully vaccinated until 13 days</i>	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)
<i>Fully vaccinated</i>	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										
<i>Partially vaccinated</i>	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)
<i>Fully vaccinated until 13 days</i>	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)
<i>Fully vaccinated</i>	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										
<i>Partially vaccinated</i>	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)

<i>Fully vaccinated until 15 days</i>	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)
<i>Fully vaccinated</i>	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
<i>Infection</i>		
<i>Partially vaccinated</i>	33.2 (32.3-34.0)	16.5 (15.2-17.8)
<i>Fully vaccinated until 13 days</i>	55.5 (53.7-57.3)	38.0 (36.9-39.0)
<i>Fully vaccinated</i>	69.8 (68.2-71.3)	54.6 (53.7-55.5)
Hospitalization		
<i>Partially vaccinated</i>	51.3 (49.9-52.7)	25.5 (23.4-27.6)
<i>Fully vaccinated until 13 days</i>	67.6 (64.8-70.1)	55.4 (53.8-56.8)
<i>Fully vaccinated</i>	86.0 (84.1-87.6)	72.5 (71.4-73.6)
ICU admission		
<i>Partially vaccinated</i>	53.7 (51.3-56.0)	27.8 (24.3-31.1)
<i>Fully vaccinated until 13 days</i>	67.2 (62.4-71.3)	56.7 (54.2-59.0)
<i>Fully vaccinated</i>	87.4 (84.3-89.9)	74.1 (72.3-75.8)
Death		
<i>Partially vaccinated</i>	48.2 (45.6-50.6)	28.8 (25.8-31.6)
<i>Fully vaccinated until 13 days</i>	70.4 (66.8-73.7)	57.9 (55.8-59.9)
<i>Fully vaccinated</i>	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								
<i>Reference period</i>	18,420	23,368	78.8	Ref	15,080	19,672	76.6	Ref
<i>Partially vaccinated</i>	20,998	27,946	75.1	50.7 (49.6-51.9)	14,484	19,182	75.5	25.5 (23.8-27.2)
<i>Fully vaccinated</i>	574	845	67.9	85.8 (84.3-87.1)	20,299	26,836	75.6	71.5 (70.6-72.4)
ICU admission								
<i>Reference period</i>	6,272	7,693	81.5	Ref	5,643	7,176	78.6	Ref
<i>Partially vaccinated</i>	7,129	9,164	77.8	52.4 (50.5-54.3)	5,291	6,875	77.0	26.9 (24.1-29.6)
<i>Fully vaccinated</i>	184	262	70.2	87.5 (85.1-89.5)	6,971	9,015	77.3%	73.2 (71.8-74.6)

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)

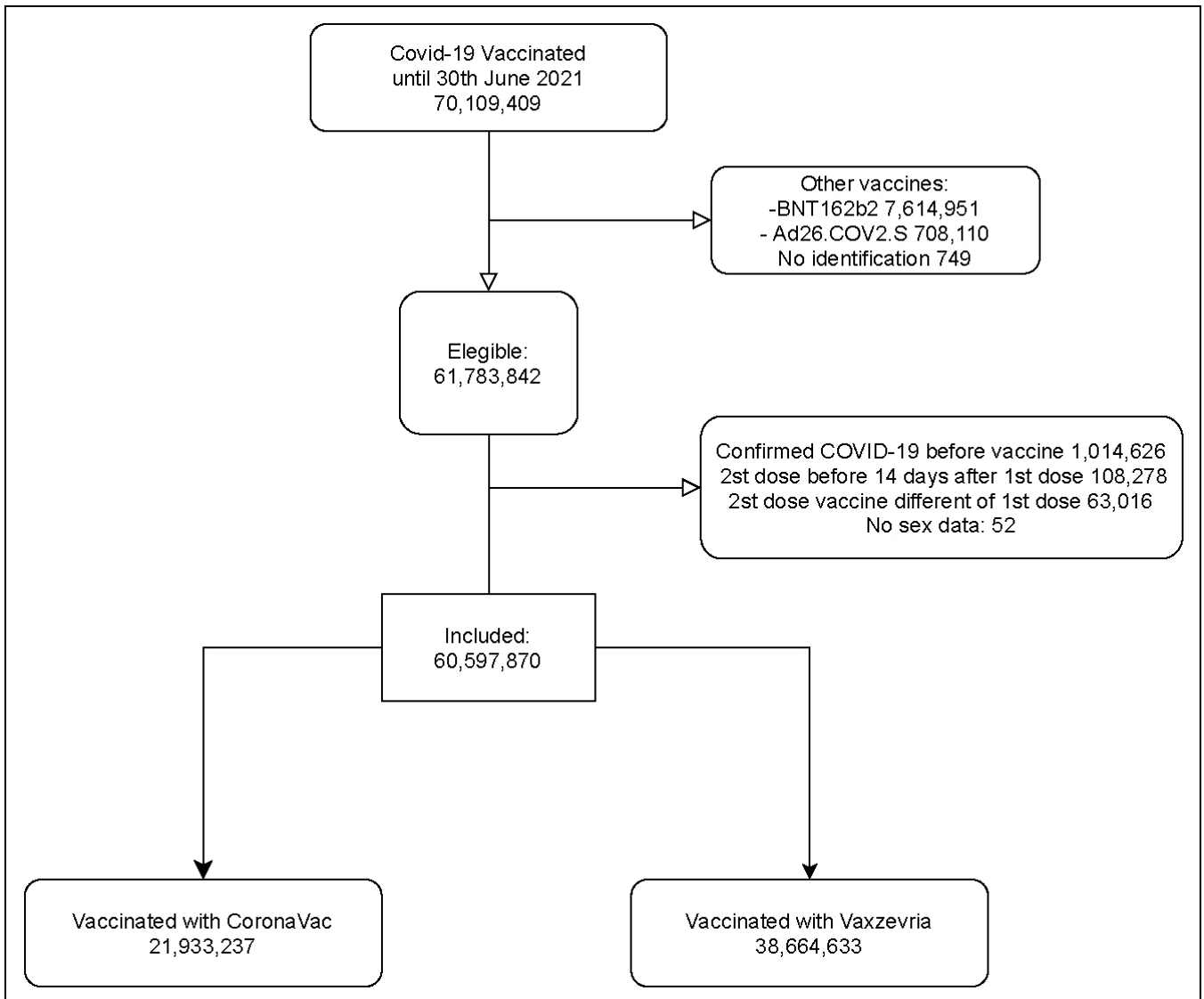
*Obtained through Cox regression model adjusted for age, sex, region of residence, month of administration of first dose and municipal deprivation level

Figures legends

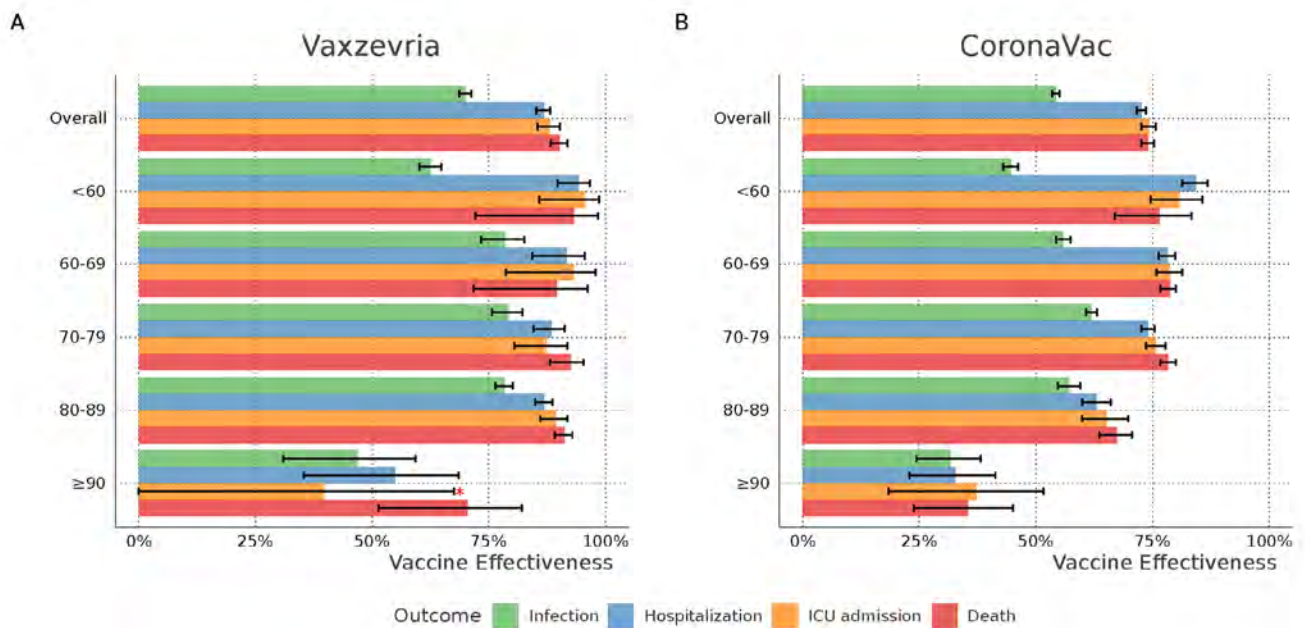
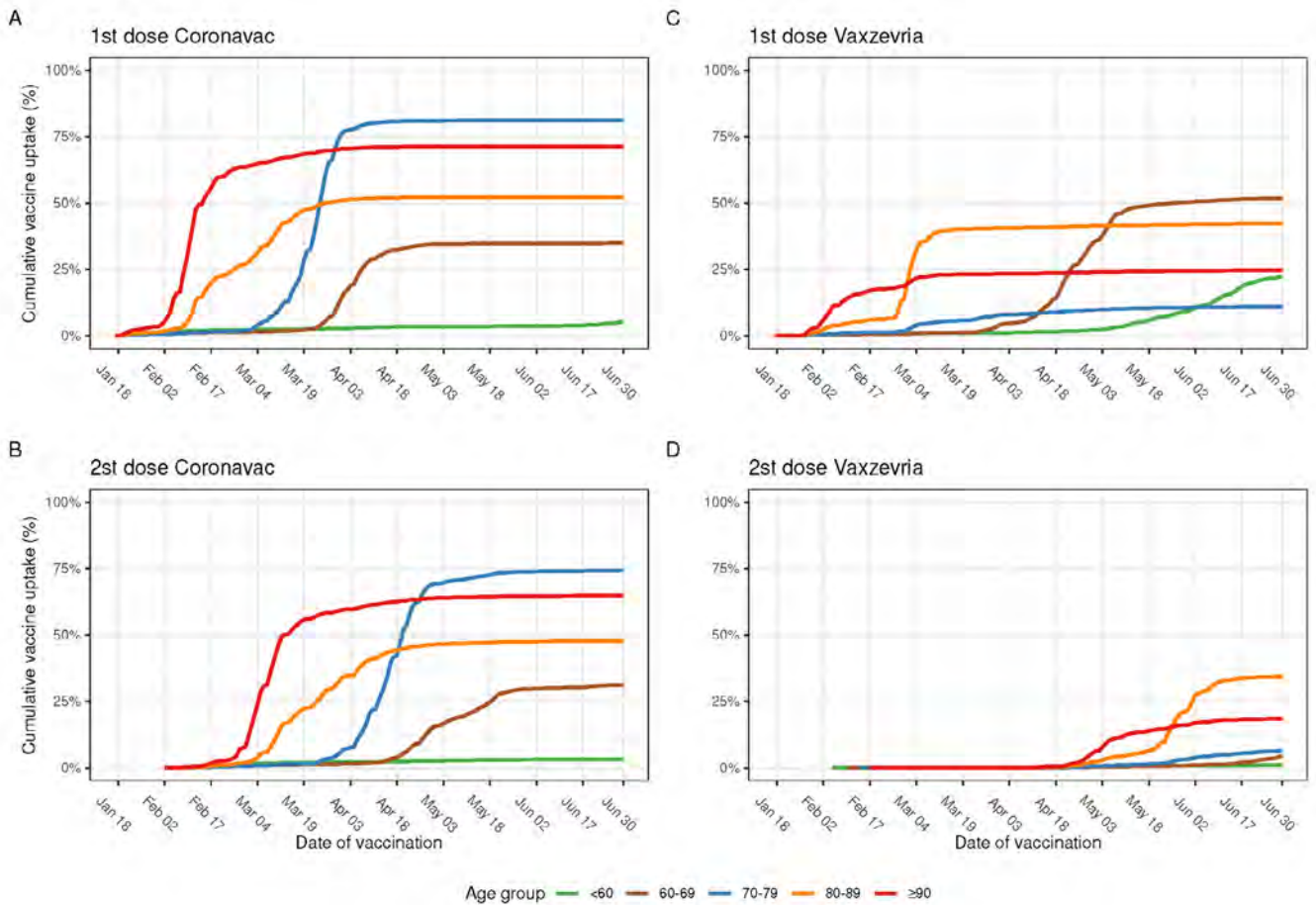
Figure 1. Flowchart of the selection of the study individuals vaccinated between 18th January and 30 June 2021. Eligible participants received at least one dose of CoronaVac or Vaxzevria vaccine between January 18 and June 30, 2021. We excluded persons with confirmed COVID-19 diagnosis in 2021 before 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.



Article



5.10. Study confirms CoronaVac efficacy against the gamma variant (P.1) among the elderly

A research published in the MedRxiv attests to the efficacy of CoronaVac, a vaccine from Butantan and the Chinese pharmaceutical company Sinovac against Covid-19, in preventing the gamma variant (P.1, Amazonian) of the SARS-CoV-2 virus in elderly people over 70 years old.

The vaccine's effectiveness against hospitalizations 14 days after the second dose was 59%, and against deaths, 71.4%. The indicator varied with the increase of age: among individuals aged 70 to 74 years old, the efficacy was 61.8% against symptomatic disease, 80.1% against hospitalizations, and 86% against deaths.

“In summary, it was evidenced that a two-dose CoronaVac vaccine regimen was effective in preventing symptomatic cases of Covid-19 and in preventing more severe clinical outcomes among the elderly facing the gamma variant,” state the authors in the article.

The work was conducted by researchers associated with the

Secretariat of Health of the State of São Paulo, the Pan American Health Organization, the University of São Paulo and the American universities of Florida and Yale, among other institutions. A total of 43,774 adults aged 70 years old or over, living in the state of São Paulo, all symptomatic for Covid-19, were investigated.

The aim of the research was to estimate the efficacy of CoronaVac against symptomatic Covid-19 in the elderly population of São Paulo State, during the wide circulation of the gamma variant between January and April of 2021.

The authors conclude that, although further research must still contribute to reaffirm the efficacy of CoronaVac against the gamma variant, results provide evidence to support the use of the vaccine in Brazil and in the other South American countries facing the spread of the SARS-CoV-2 gamma variant.

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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

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Keywords

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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 systems²⁸ 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 $\geq 80\%$ 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 variants²⁰ 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 ($p_{\text{interaction}} = 0.05$)(Figure 3). The same pattern was observed for hospitalisations ($p_{\text{interaction}} = 0.04$) and deaths ($p_{\text{interaction}} = 0.19$), 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% , $p_{\text{interaction}} = 0.12$) 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 Finland³⁸ 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 Gamma²⁰ 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/julicroda/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.

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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.

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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.

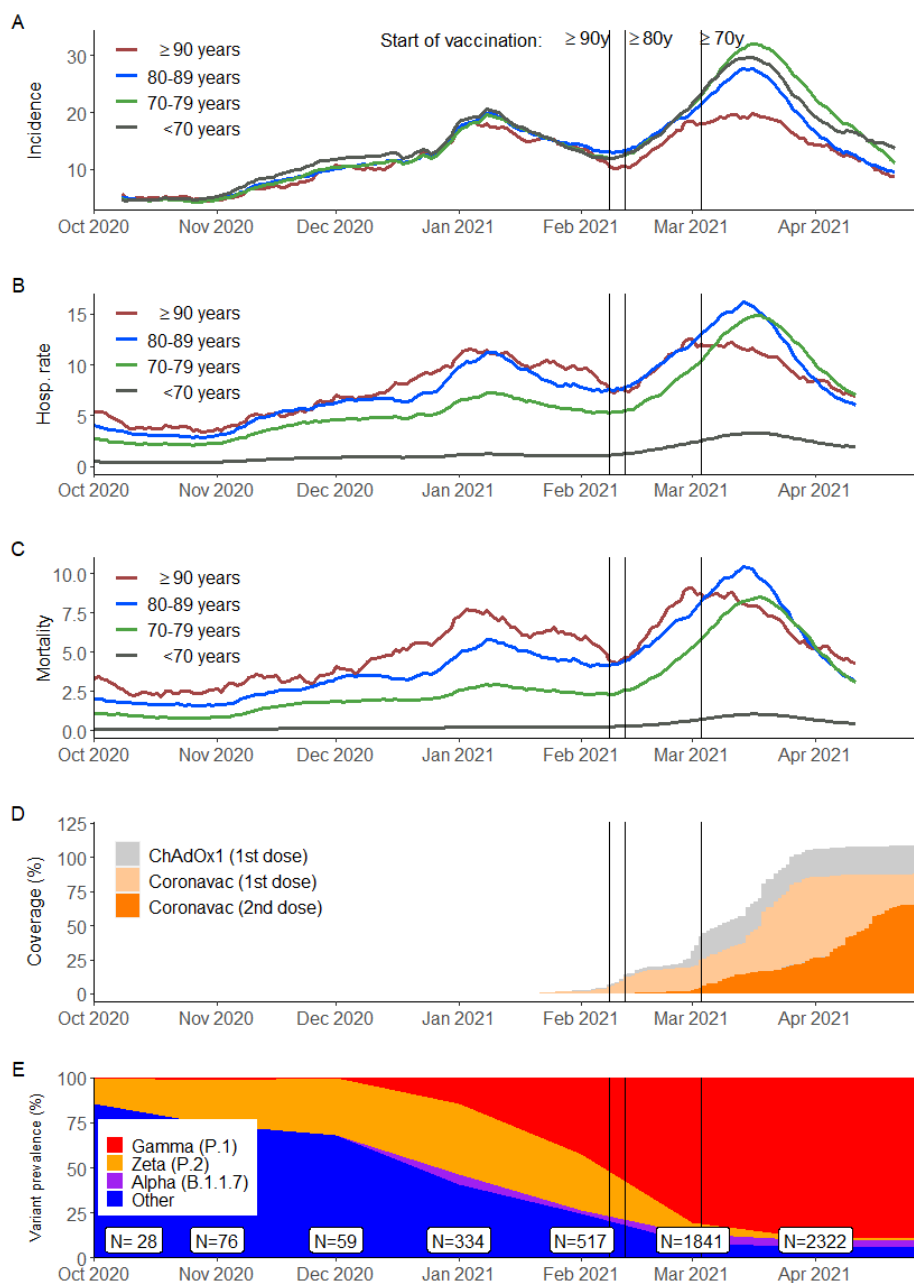


Figure 2. Flowchart of the identification of the study population from surveillance databases and selection of matched cases and controls.

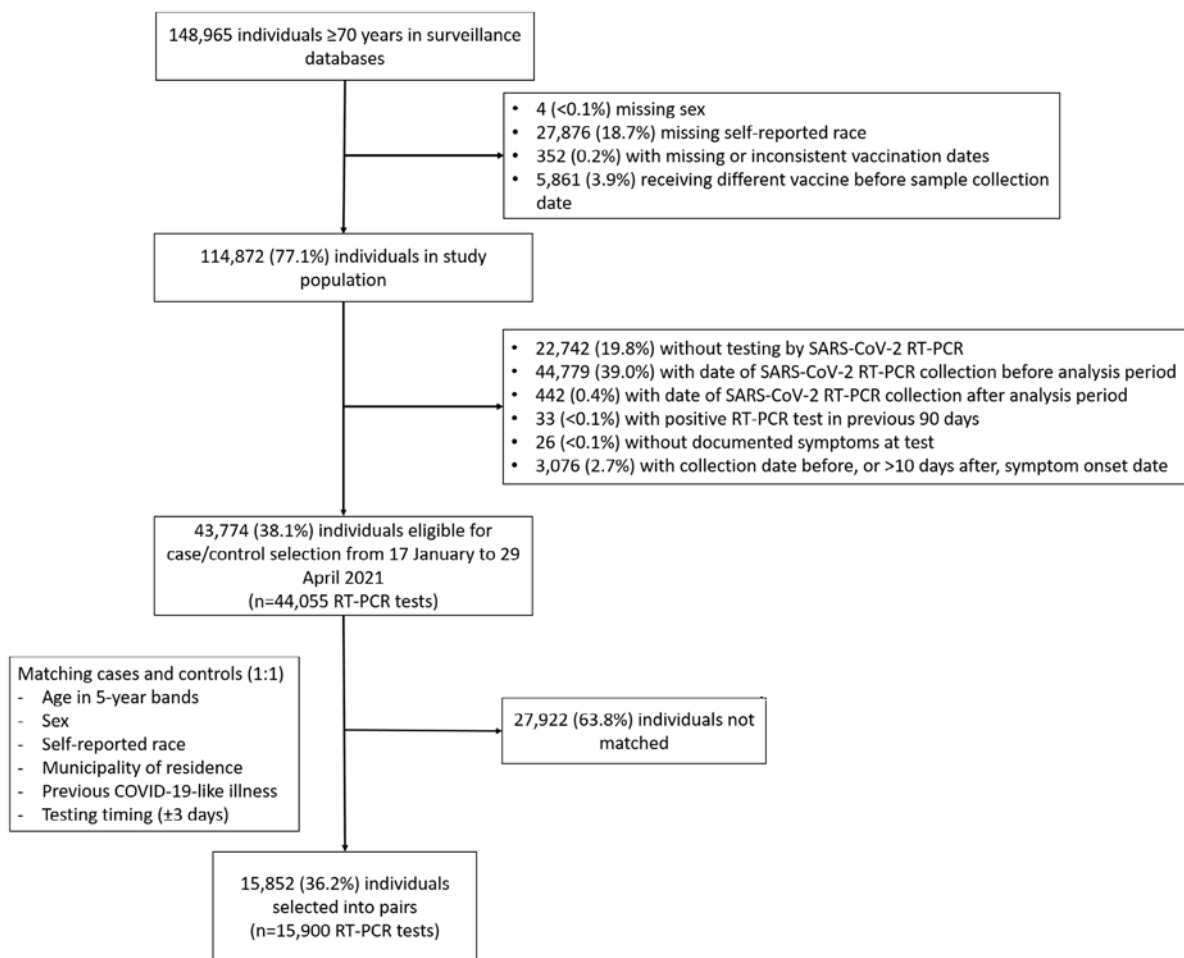


Figure 3. Adjusted vaccine effectiveness during the period ≥ 14 days after the second CoronaVac dose for subgroups of adults ≥ 70 years of age. Estimates of vaccine effectiveness were obtained from a conditional logistic regression model that included covariates of age and the number of comorbidities and incorporated an interaction term between the category of interest and the period ≥ 14 days after the second CoronaVac dose.

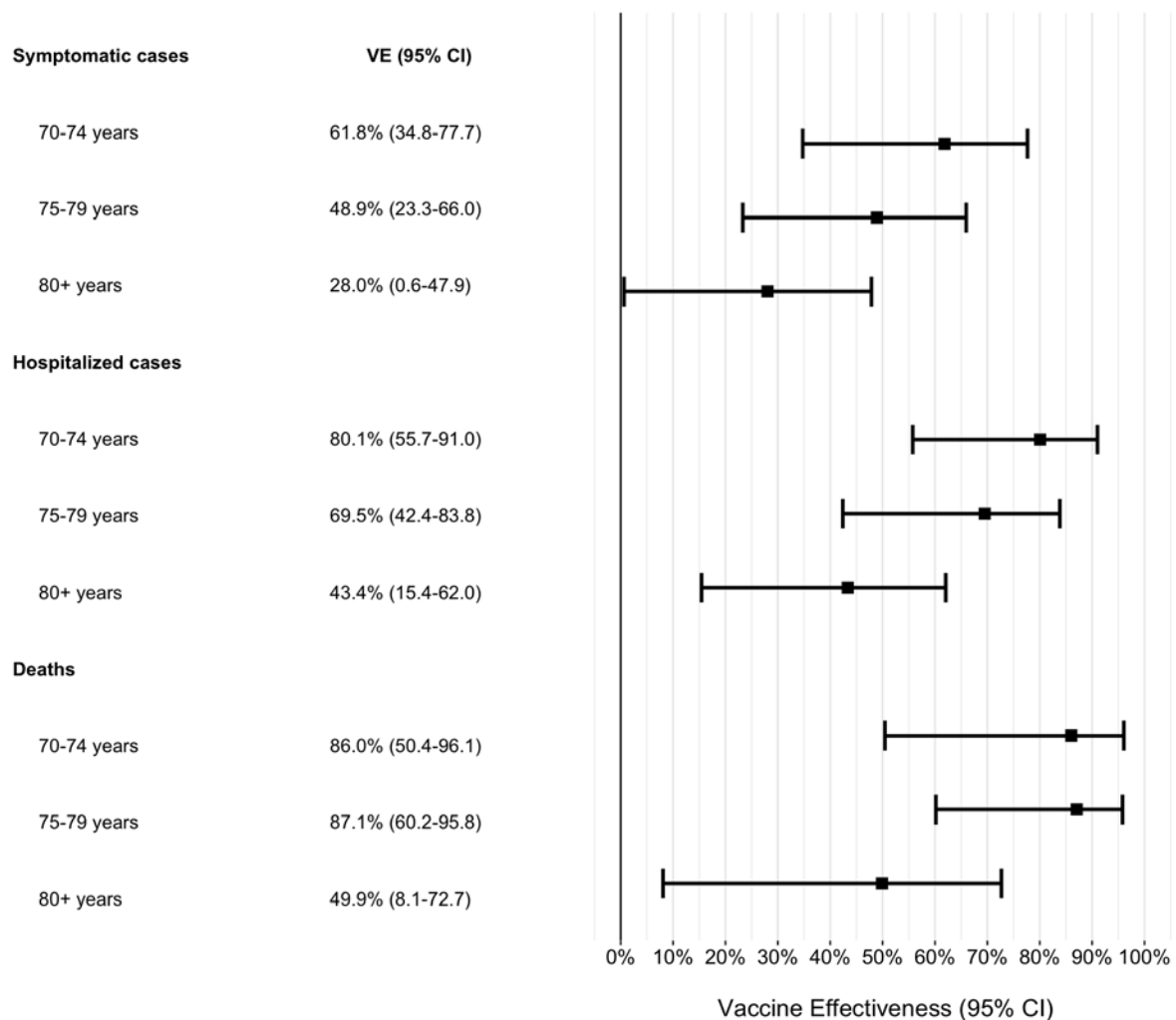


Table 1. Characteristics of adults ≥70 years of age who were eligible for matching and selected into case-test negative pairs.

Characteristics*	Eligible cases and controls		Matched pairs	
	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.

Symptomatic COVID-19 (n=15,900)	Unadjusted Analysis			Adjusted Analysis [^]		
	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

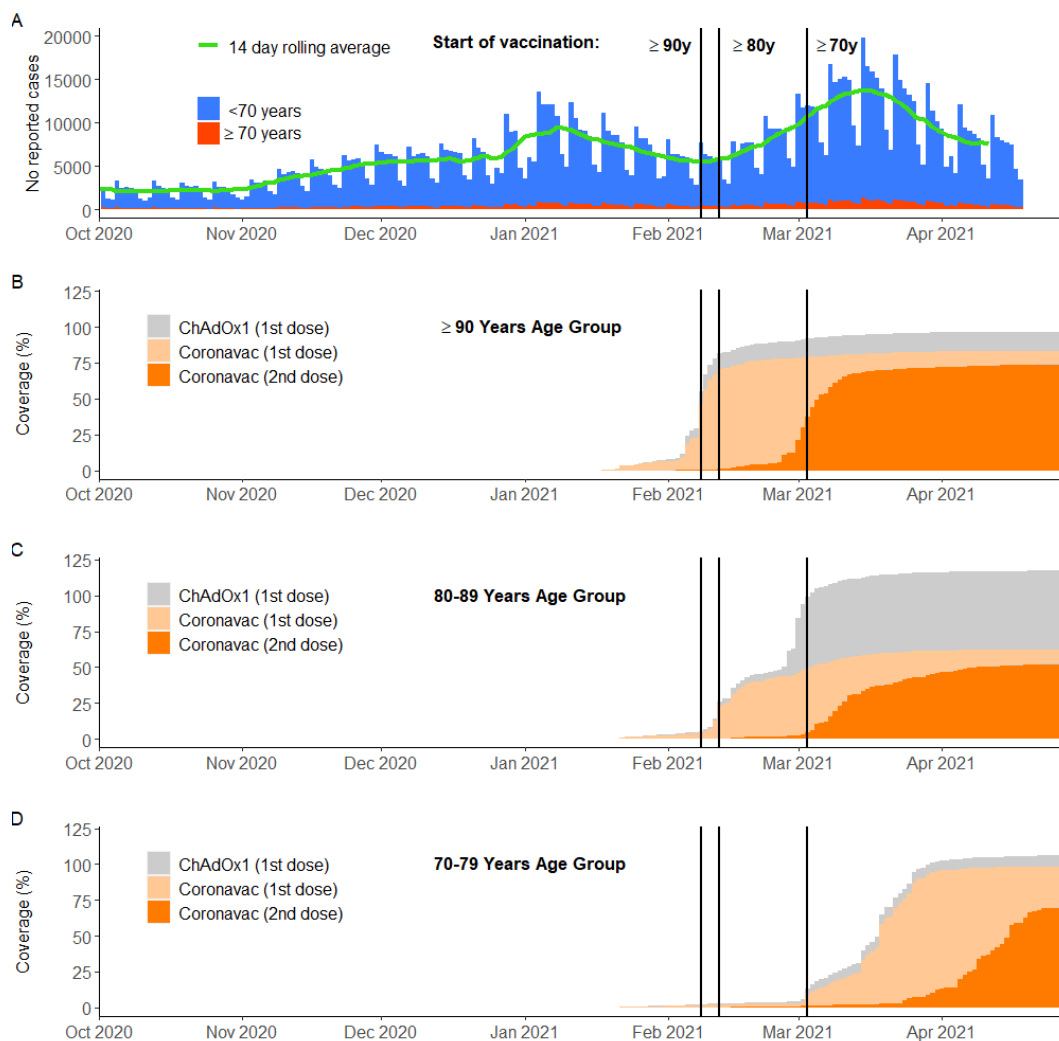
Supplement to: Effectiveness of the CoronaVac vaccine in the elderly population during a Gamma variant-associated epidemic of COVID-19 in Brazil: A test-negative case-control study

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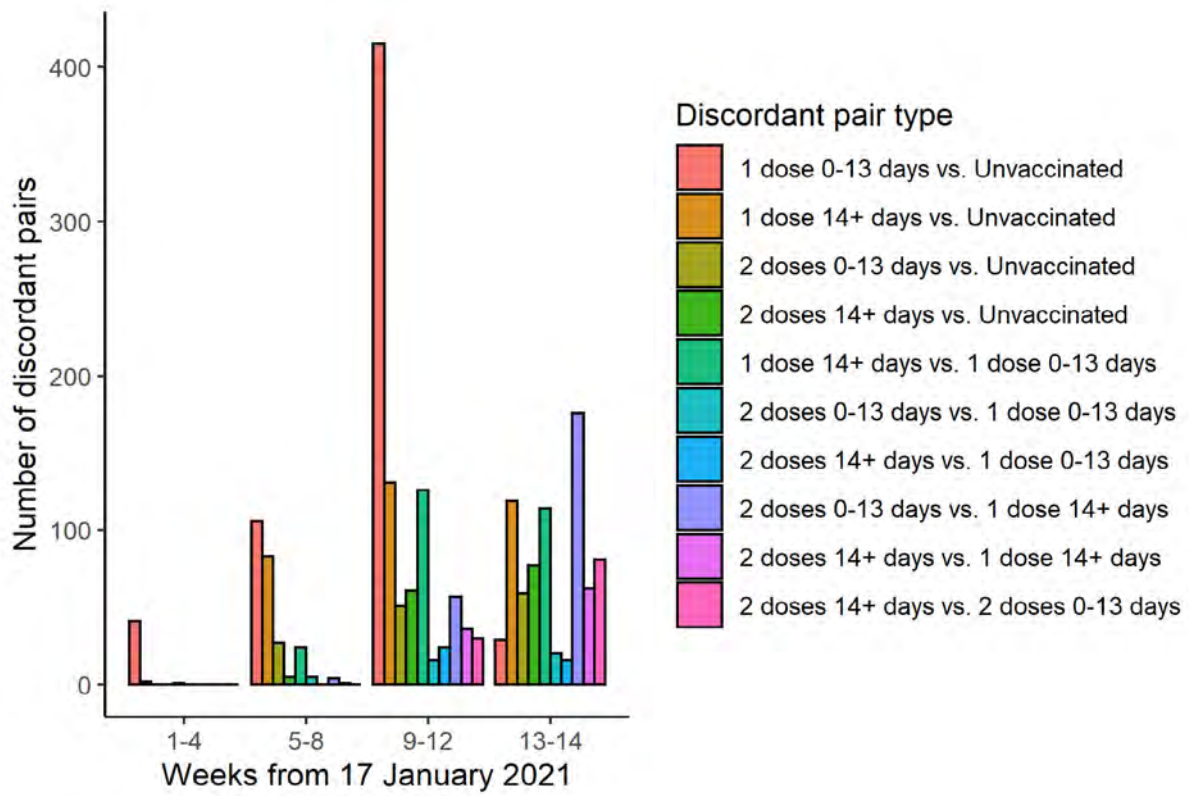
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Supplementary Figure 1. Daily cases and vaccine coverage by age.

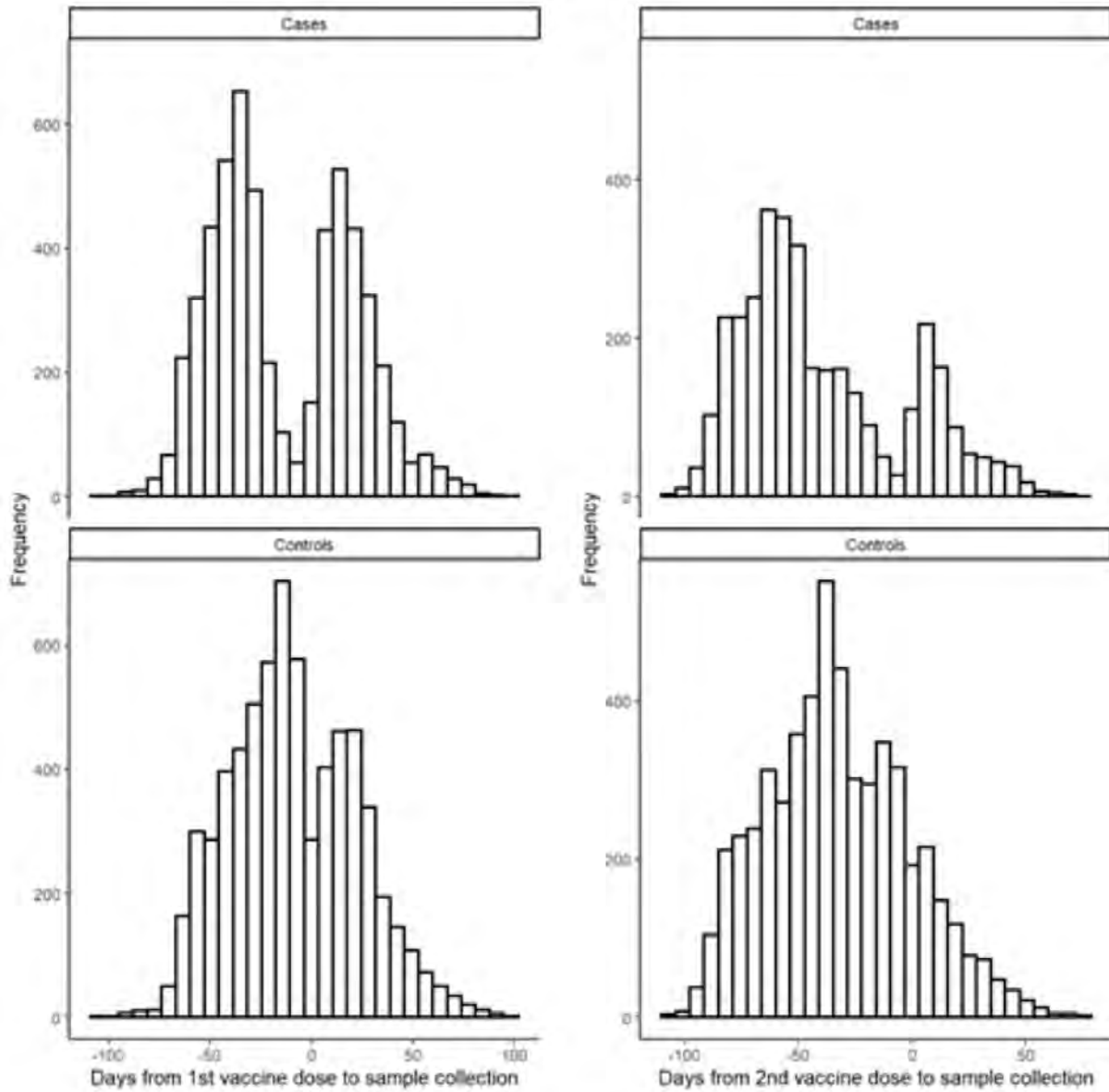
Panel A shows the daily cases of reported COVID-19 from Mar 15, 2020 to Apr 29, 2021 in São Paulo State, Brazil, with the green line representing the 14-day rolling average of counts. Panels B, C and D show the cumulative vaccination coverage for age groups >90y, 80y-89y, and 70y-79y, respectively. Population 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.

Characteristics*	Eligible cases and controls		Matched pairs	
	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.

Characteristics*	Eligible cases and controls		Matched pairs	
	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)	

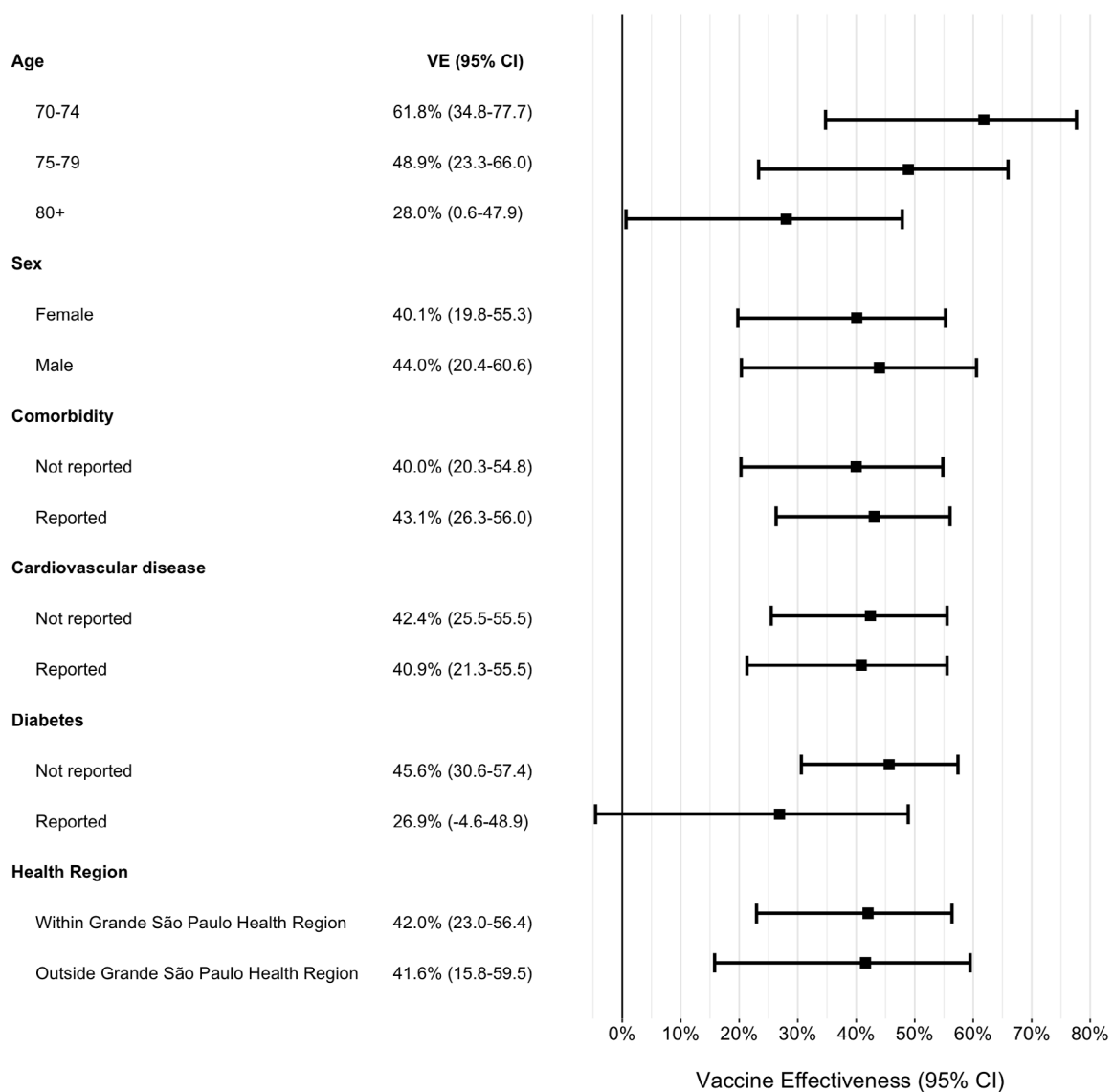
Supplementary Table 5. Estimated effectiveness of CoronaVac ≥ 14 days after the second dose, in subgroups of adults ≥ 70 years of age.

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. P.1. 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

ChAdOx1 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
- 14-27 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 ChAdOx1 for 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 ChAdOx1 by 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 and 0-13 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.

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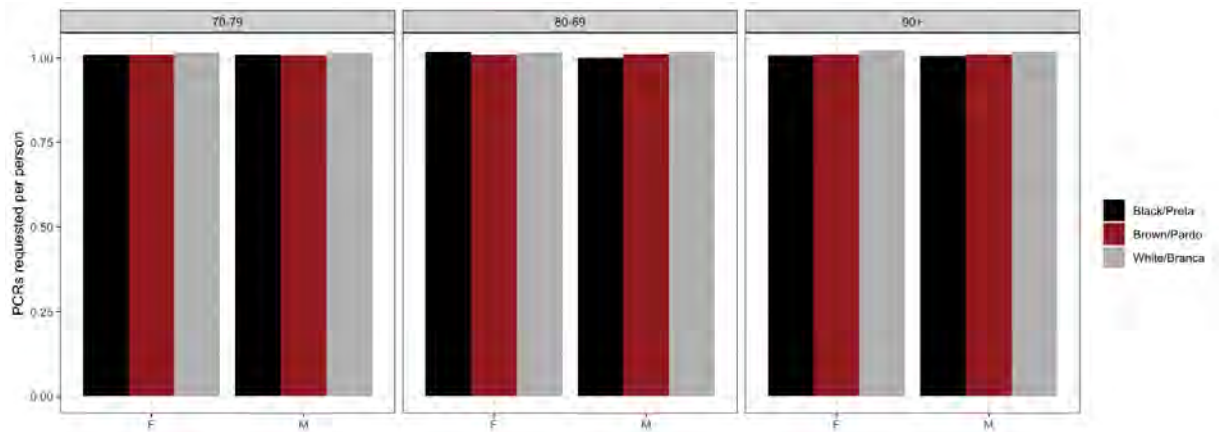


Figure 1: PCR testing rate by age, sex and self-reported race (from data extracted on April 07, 2021)

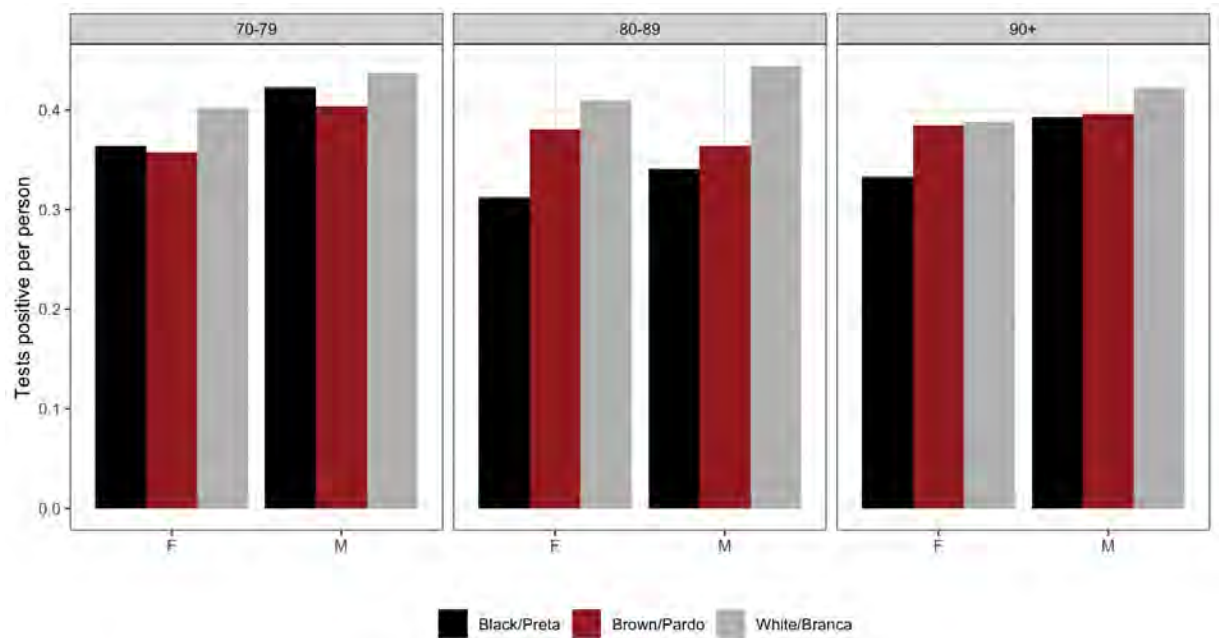


Figure 2: PCR positive testing rate by age, sex and self-reported race (from data extracted on April 07, 2021)

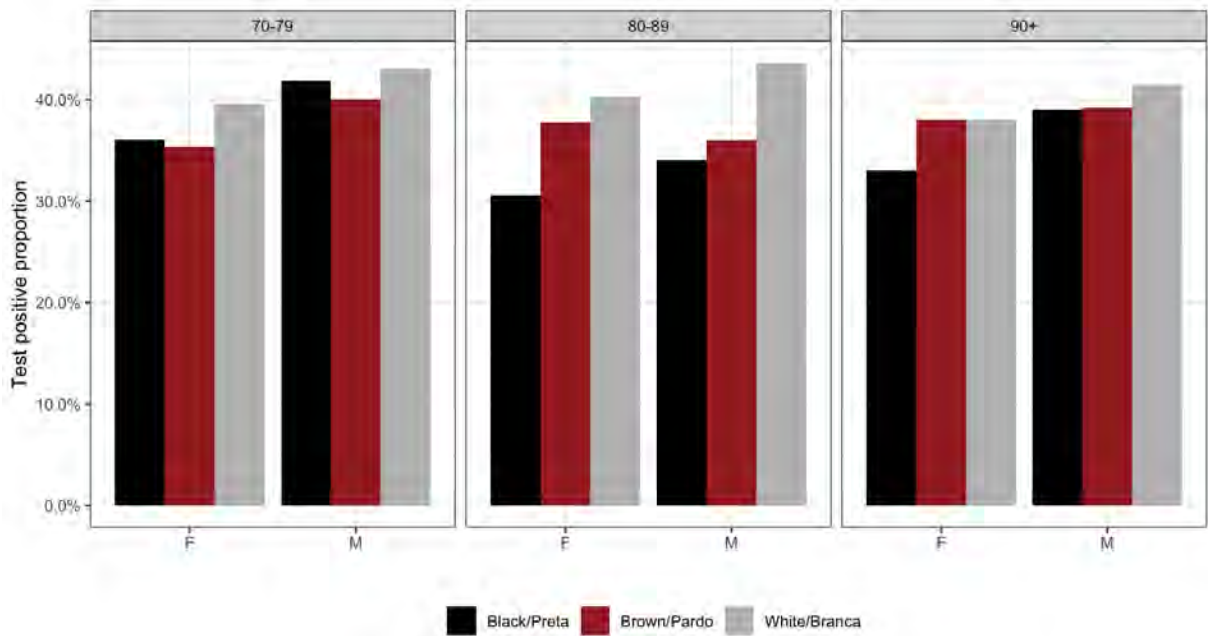


Figure 3: PCR positive proportion by age, sex and self-reported race (from data extracted on April 07, 2021)

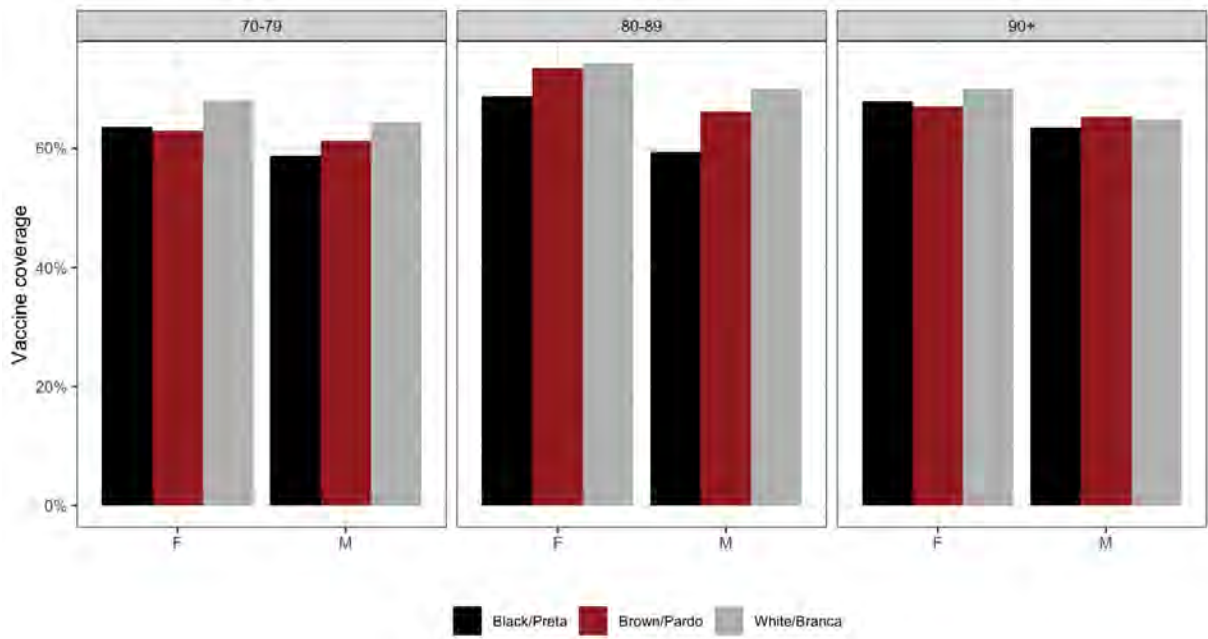
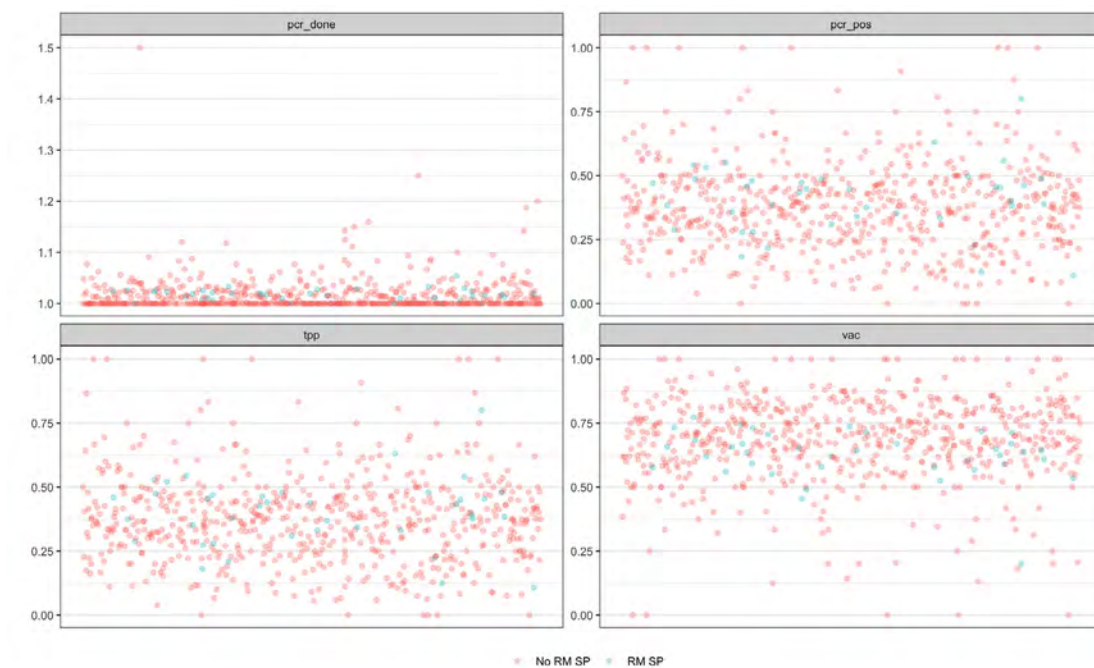


Figure 4: Vaccine coverage by age, sex and self-reported race (from data extracted on April 07, 2021)

Panel A. Indicators by Municipality



Panel B. Indicators by Municipality and Race

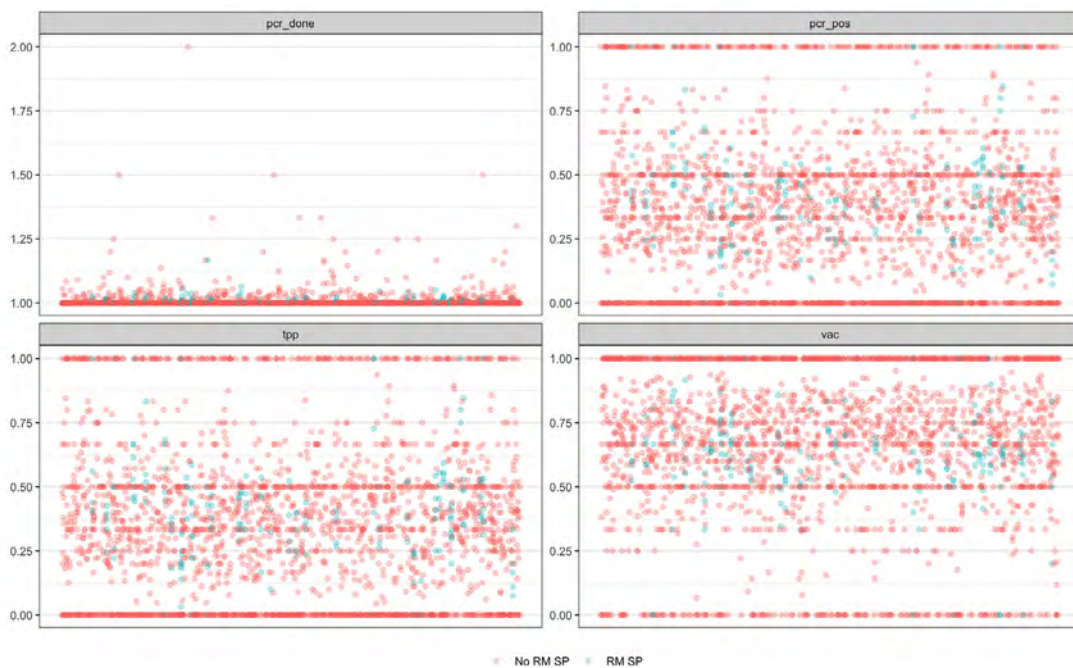
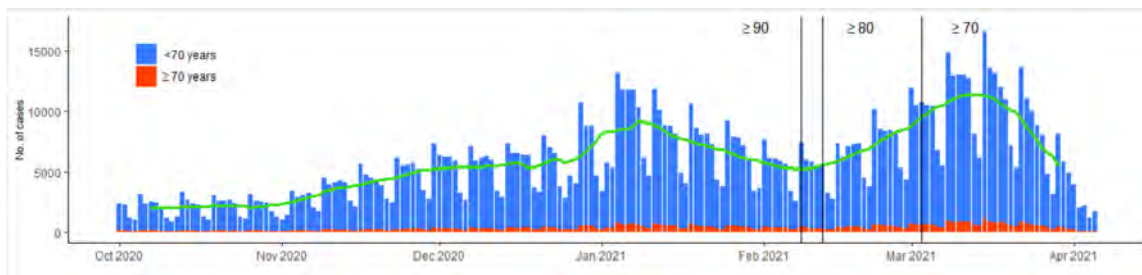


Figure 5: PCR testing rate (pcr_done), PCR positive testing rate (pcr_pos), positivity proportion (tpp) and vaccine coverage (vac) by each municipality (A) and municipality and race (B). RM SP denotes metropolitan area of São Paulo city (from data extracted on April 07, 2021)

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.

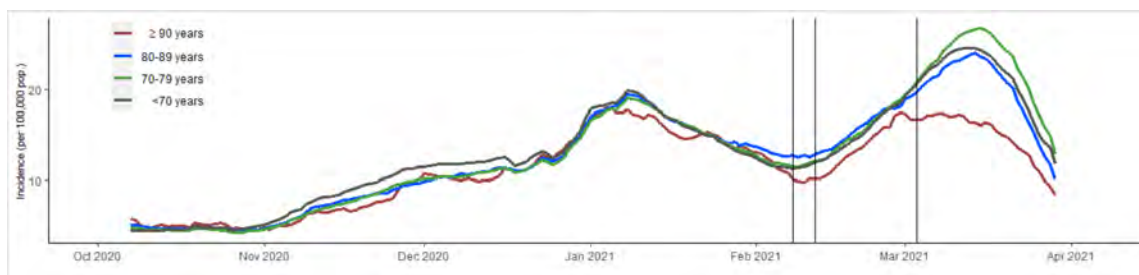


Table 1: Table of planned analyses

Analysis	Exposure	Outcome
CoronaVac		
Primary outcome, primary exposure	Two-dose regimen of CoronaVac in the period starting 14 days after administration of the 2 nd 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 2 nd dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of CoronaVac, in the period starting 14 days after administration of the 1 st dose	
Primary outcome, bias indicator	One-dose regimen of CoronaVac, in the period 0-13 days after administration of the 1 st dose	
ChAdOx1		
Primary outcome, primary exposure	One-dose regimen of ChAdOx1 in the period starting 28 days after administration of the 1 st 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 2 nd dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of ChAdOx1 in the period 0-13 days after administration of the 1 st dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of ChAdOx1, in the period starting 14-27 days after administration of the 1 st dose	
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of ChAdOx1, in the period starting 0-13 days after administration of the 2 nd dose	
Primary outcome, bias indicator	One-dose regimen of ChAdOx1, in the period 0-13 days after administration of the 1 st dose	

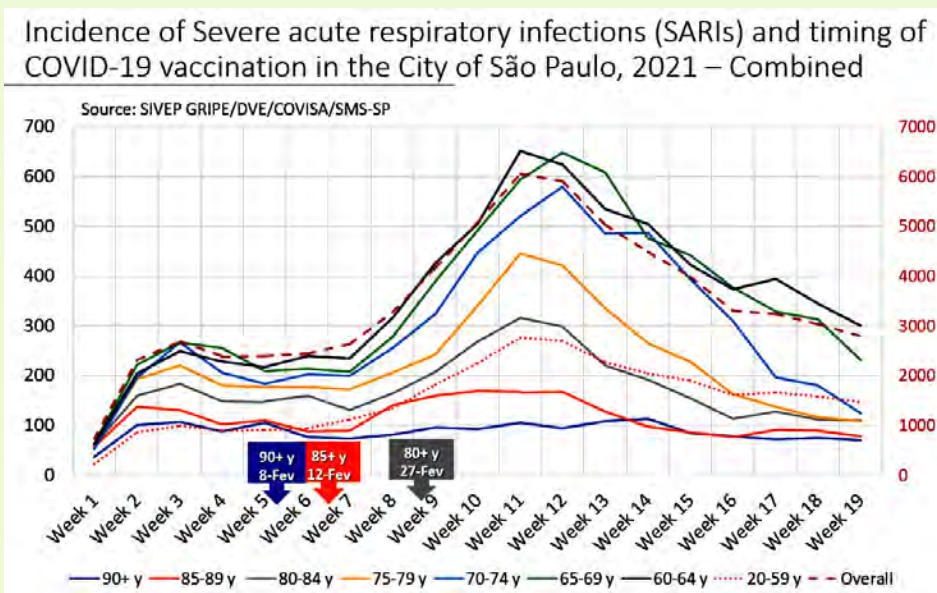
5.11. CoronaVac is associated with the decrease of Covid-19 mortality among elderly people, studies show

Studies conducted by researchers from Brazil, United States and Spain have shown that the administration of CoronaVac, the vaccine from Butantan against Covid-19, led to a decrease in hospitalization and deaths from SARS-CoV-2 among elderly patients, including in settings where the gamma variant (P.1) of the new coronavirus predominates.

According to the article “Estimating the early impact of vaccination against Covid-19 on deaths among elderly people in Brazil”, the escalation of vaccination among the elderly in the country is associated with a considerable drop in mortality among the elderly

compared to younger people. Between January–February (when few elderly people had taken the second dose) and April 2021, the drop in the number of deaths in the population over 80 years old was from 25% to 13%.

Between the first epidemiological week and April 22, 2021, 171,517 deaths were attributed to Covid-19 according to the Ministry of Health’s Mortality Information System. The following graph shows that there is a clear acceleration in deaths starting in week 9 (early March), when the P.1 variant begins to predominate in Brazil.



Between epidemiological weeks 13 and 14 (in April, when about 10 million people had received the second dose), there begins a slowdown in the number of deaths, especially in people over 70 years old. In the graph, it is evident that there was no increase in the number of positive cases in the group over 90 years old, which shows that the vaccine has become effective in containing the force of infection of the virus in this age group.

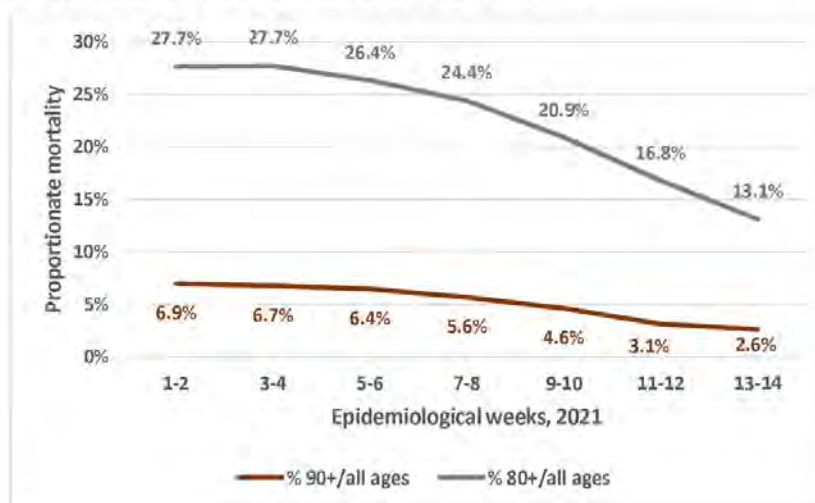
Furthermore, the study “Effectiveness of the CoronaVac vaccine in the elderly population during a Gamma variant-associated epidemic of Covid-19 in Brazil”, conducted between January and April 2021, with 15,000 cases of people over 70 years old in the

state of São Paulo, showed that the vaccine’s effectiveness in a P.1 variant context increases over time and does not vary significantly from the vaccines overall effectiveness, being 49.4% 21 days after the second dose. It is higher, however, in the younger elderly: those aged 70 to 74, the efficacy is 61.8%.

Effectiveness data from studies using the vaccine routinely may vary, and therefore should be interpreted with caution. Not to mention that the researchers vary from methodological point of view and analyze different epidemiological moments.

It should be noted that the prediction of the study effectiveness is based on the relation between vaccination numbers and numbers

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.



of confirmed cases and deaths from Covid-19. The research is not based on clinical hospitalization indicators. The primary aim of CoronaVac is to reduce the number of deaths and hospital admissions, reducing the impact of the disease on the deaths and the healthcare system.

Studies conducted in Brazil and in other countries have shown that CoronaVac is effective against the new variants, like P.1 and P.2, and that it protects all age groups, including the elderly group, against Covid-19 mortality. But it is important to note that no vaccine prevents a person

from becoming infected with the coronavirus.

Another relevant point is that any vaccine generates a lower immune response in older people. This does not mean that they are less protected against the disease, but rather that the body responds less to a novel antigen - a characteristic that is not related to the effectiveness of the vaccine itself, but to the natural processes of the immune system.

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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

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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 15, 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.

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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

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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.

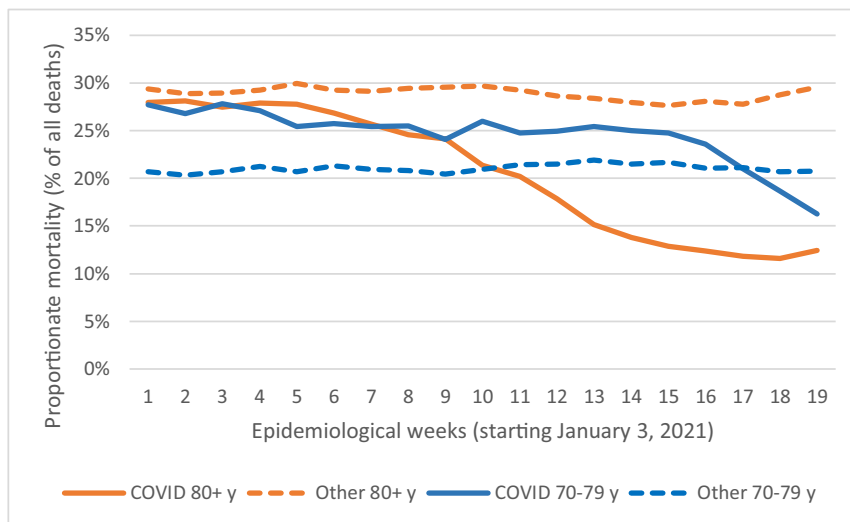


Fig. 1. Proportionate mortality of individuals aged 70–79 and 80 or more years due to COVID-19 and all other causes, relative to deaths due to the same causes at all ages by epidemiological weeks, Brazil, 2021.

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+

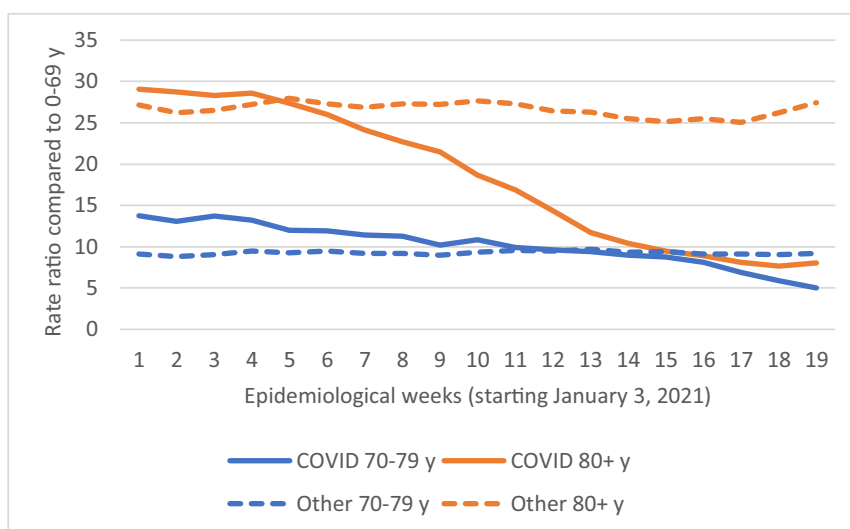


Fig. 2. Mortality rate ratios: mortality rates at ages 70–79 and 80+ years divided by mortality rate at ages 0–69 years by epidemiological weeks, Brazil 2021.

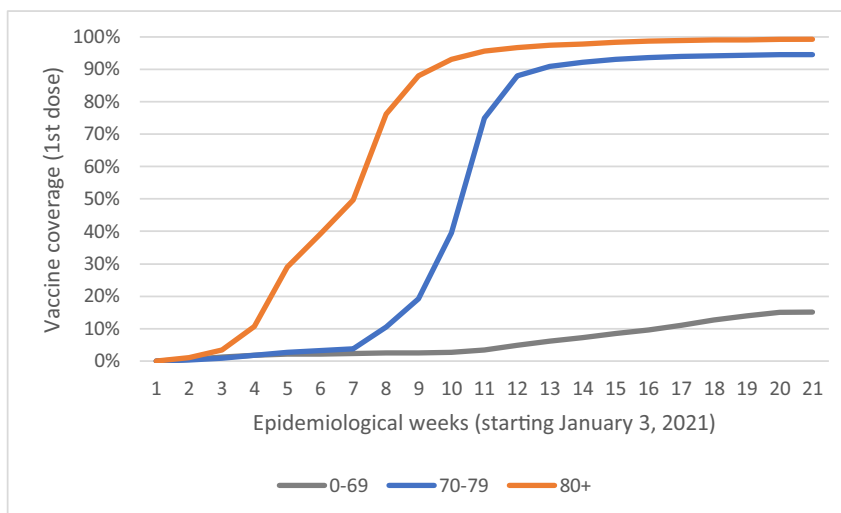


Fig. 3. Covid-19 vaccination coverage (first dose) by age group by epidemiological week, Brazil, 2021.

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 disproportionately 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.

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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.

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CoronaVac

What science proves

6 ■ It protects children and adolescents

6.1. CoronaVac adverse event rate in children and adolescents in Brazil is less than one case per 100,000 doses applied

The incidence rate of adverse events in children and adolescents who took CoronaVac in Brazil is 0.76 per 100,000 doses applied, according to notifications received by the Pharmacovigilance of Instituto Butantan. That is, the notification of adverse events among those under 17 years of age who took the Butantan-Sinovac vaccine was less than one case per 100,000 doses administered, which proves the very low reactogenicity of CoronaVac in the age group from 6 to 17 years. Adverse events were spontaneously reported to the Pharmacovigilance of Instituto Butantan, which carried out the survey with data up to the end of July.

The survey takes into account the more than 13 million doses of CoronaVac applied in this age group until the period surveyed - 11.05 million in children aged 6 to 11 years and 2.02 million in adolescents aged 12 to 17 years.

The National Immunization Program of the Brazilian Ministry of Health recommends the use of CoronaVac in two doses, with a 28-day interval, for children over 3 years of age, adolescents and adults. As the authorization for the use of CoronaVac in children under

5 years of age was authorized by the Brazilian Health Regulatory Agency (ANVISA) only on July 13, the survey has data on vaccination of children from 6 years of age, whose authorization from ANVISA occurred in January 20.

Adverse events in children aged 6 to 11 years

According to the Butantan survey, the occurrence of adverse events in children aged 6 to 11 years is even lower than the general average. The total of 64 cases reported in children in this age group represents only 0.58 adverse events for every 100,000 doses applied.

When this data is refined, the incidence rate of non-serious adverse events is 0.38 per 100,000 doses applied. The events with the highest incidence are: fever (incidence rate of 0.09 per 100,000 doses), vomiting (incidence rate of 0.04 per 100,000 doses), pain at the injection site (0.03 per 100,000 doses) and headache (0.03 per 100,000 doses).

The incidence rate of serious adverse events is 0.20 per 100,000 doses applied. The most incident events

were hypersensitivity and syncope (fainting), with an incidence rate of 0.04 per 100,000 doses.

Adverse events in adolescents aged 12 to 17 years

Adverse events in adolescents aged 12 to 17 years who took both doses of CoronaVac are quite rare, according to data from Butantan's Pharmacovigilance collected in the same period. Thirty-six adverse events (33 non-serious and three serious) were reported among all 2 million vaccinated adolescents, which is equivalent to a rate of 1.78 adverse events per 100,000 doses administered to this population.

Among the non-serious adverse events reported, there were oropharyngeal pain, hypersensitivity (both with rates of 0.20 per 100,000 doses applied) and post-vaccination cough (rate of 0.15 per 100,000 doses applied). There were also reports of malaise, fever, sneezing (rate of 0.1 adverse event per 100,000 doses applied), in addition to pain at the injection site, nasal congestion and itching at the injection site (rate of 0.05 adverse event per 100,000 doses applied).

Among the three serious adverse events reported in this population, which were not related to vaccination, one was intussusception (intestinal obstruction), one was enterocolitis and one was not specified by the reporter (rate of 0.05 adverse event per 100,000 doses applied).

“The total number of adverse events spontaneously received by the Pharmacovigilance of Instituto Butantan compared with the estimate of individuals exposed to the vaccine show that CoronaVac is a very safe product for these audiences,” says the scientific researcher and qualified person for Pharmacovigilance at Instituto Butantan, Vera Gattás.

6.2. CoronaVac is 59% effective against hospitalizations in children and adolescents aged 6 to 17 years, Brazilian study shows

A study conducted by the Oswaldo Cruz Foundation (Fiocruz), published in the journal *Nature Communications*, showed that CoronaVac was able to protect children and adolescents aged 6 to 17 years against severe cases of Covid-19 during the omicron outbreak. The estimated effectiveness was 59.2% against Covid-19 hospitalizations. Scientists analyzed data from almost 200,000 children, immunized between January and April 2022, after approval of the Butantan vaccine by the Brazilian Health Regulatory Agency (ANVISA).

Data were obtained from e-SUS Notifica, the national surveillance system for RT-PCR and antigen tests for Covid-19 infection, from the Influenza Epidemiological Surveillance Information System (SIVEP-Gripe) and from the Information System of the National Immunization Program (SI-PNI).

It is worth mentioning that all vaccines have shown reduced efficiency against infection by the omicron variant, which is more transmissible, but the vaccines still prevent serious conditions and death. “These findings are also in line with previous studies in adult and adolescent populations that have shown a significant reduction in vaccine effectiveness against

Omicron compared with other variants,” the authors state.

Similar results were observed in Chile, which has been vaccinating children since last December. The effectiveness of the immunizing agent was evaluated in 500,000 children aged 3 to 5 years, also during the omicron period. CoronaVac also protected 69% against admission to Intensive Care Units (ICU) and 64.6% against hospitalizations.

Chilean pharmacovigilance data also showed that CoronaVac was the safest vaccine for children and had the lowest rate of adverse events recorded, corresponding to 0.01% of the total doses administered. Together with a series of studies, this evidence served as basis for the recent expansion of vaccine use for the 3-5 age group, approved by ANVISA on July 13. Other countries such as China, Colombia, Thailand, Cambodia, Ecuador and Hong Kong SAR already vaccinate this population with CoronaVac.

Vaccination is the only way to protect children against Covid-19, which has caused two deaths a day in children under 5 since the beginning of the pandemic, according to a survey by Fiocruz.

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Vaccine effectiveness of CoronaVac against COVID-19 among children in Brazil during the Omicron period

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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 children³, 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 multi-system inflammatory syndrome in Children (MIS-C), long-COVID syndrome^{3,4} and downstream effects of COVID-19, including social isolation and interruption in education⁴. 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, 2022⁵, 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

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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 ethnicity (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 variant⁶. 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 hospitalisation⁷. 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 status¹⁴. 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)

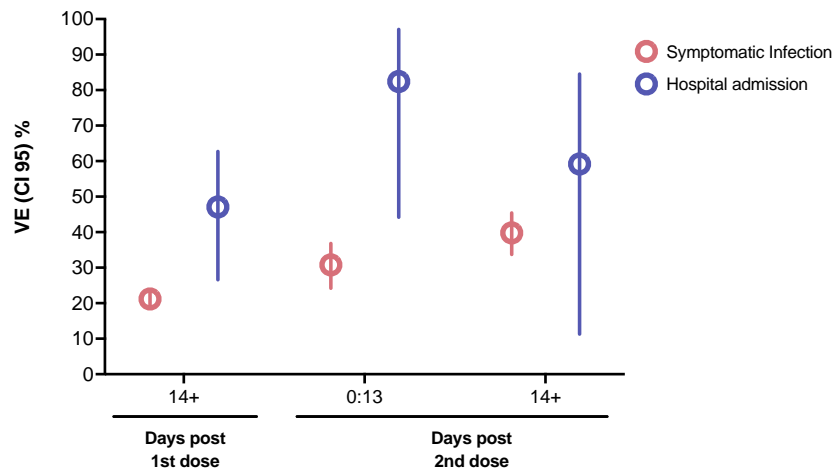


Fig. 1 | Vaccine Effectiveness for symptomatic infection and hospital admission among children aged 6–11 vaccinated with CoronaVac. The dots represent the adjusted vaccine effectiveness (VE; 1- adjusted odds ratio) estimates (sample $n = 197,958$), with error bars indicating the corresponding 95% Wald's 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 test²². The study population comprised children 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 package²⁶. 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 imputations²⁷. 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.

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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

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6.3. CoronaVac induces antibodies in more than 90% of children, including those with comorbidities, Chilean study shows

A survey from Chile once again confirmed the immunogenicity of CoronaVac in children and adolescents, with seroconversion reaching 91.8% one month after the second dose. The vaccine performed even better in those with comorbidities such as obesity, reaching 97.4% in this group. The study was published in the International Journal of Infectious Diseases and conducted by the country's Ministries of Health and Education and the School of Medicine of the University of Chile. The country has been administering CoronaVac to children over 3 years of age since December 2021 and, at the end of August, approved the vaccine for children over 6 months of age.

Scientists evaluated the seroconversion of IgG antibodies in about 2,000 children and adolescents aged 6 to 18 years, students from 24 schools located in the three most populous regions of Chile. In the analyzed group, 173 children had comorbidities such as obesity, chronic lung disease, cardiovascular disease, hypertension, diabetes and cancer.

The percentage of children who produced antibodies against SARS-CoV-2 (seroconversion) was 91.8% one month after the

second dose. Analyzing individuals separately according to age group, sex, location and comorbidities, seroconversion remained high, above 85%, in most groups.

The vaccine, recently approved in Brazil for the age group of 3 to 5 years, and which has been applied to the group from 6 to 17 since January, has already had its safety proven in several studies. Another study, also from Chile, showed that CoronaVac has the lowest rate of adverse events among the vaccines administered to children in the country, with only 10.67 notifications per 100,000 doses - 0.01% of the total doses. The vaccine also has the lowest rate of adverse events in adults.

CoronaVac's efficiency against severe cases has also been demonstrated in real-world studies. In Brazil, a recent study by the Oswaldo Cruz Foundation (Fiocruz) recorded an effectiveness of 59.2% against hospitalizations for Covid-19 in those aged 6 to 17 years. In Chile, in children aged 3 to 5 years, the effectiveness was 69% against admission to Intensive Care Units (ICU) and 64.6% against hospitalization, even during the omicron outbreak.

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Short Communication

SARS-COV-2 IgG positivity in vaccinated and non-vaccinated Chilean children: a national cross-sectional study in schools

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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).

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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.

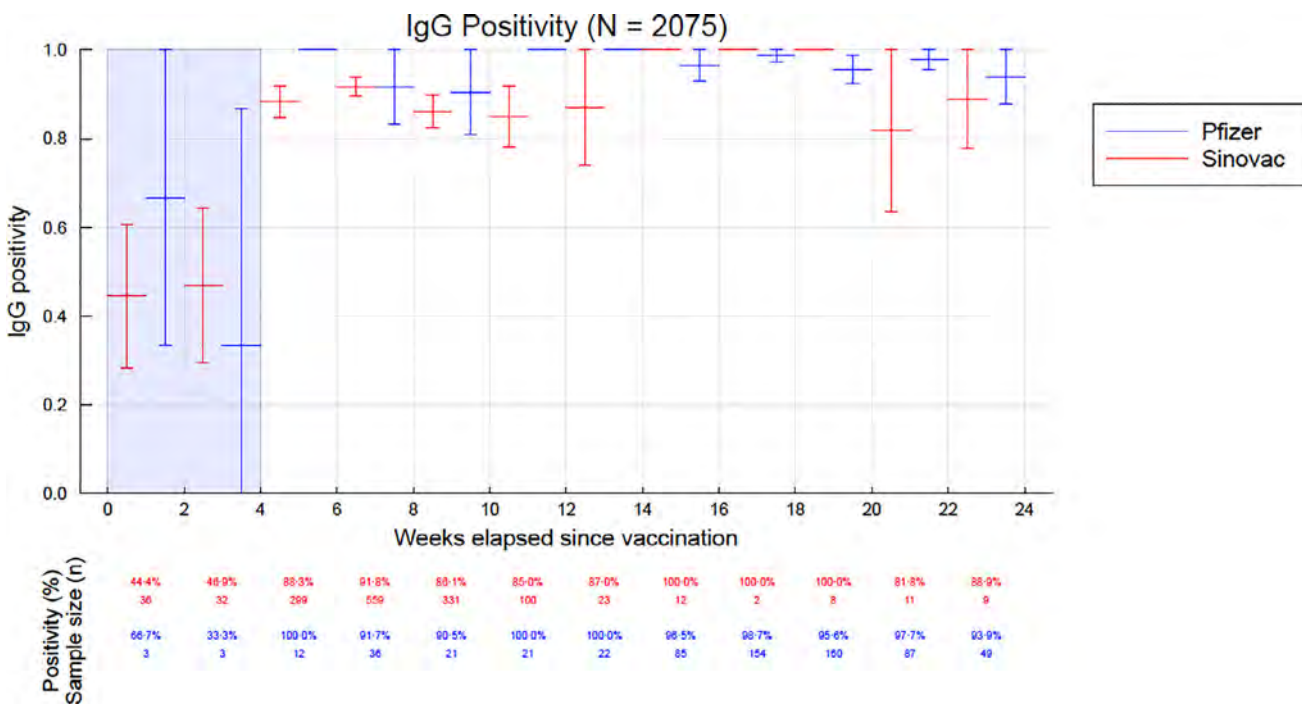


Fig. 1. Seropositivity one to four weeks after first dose (light blue-shaded region) or after second dose for recipients of Sinovac or Pfizer vaccines with no prior positive PCR result.

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.

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6.4. CoronaVac has the lowest rate of adverse events among Covid-19 vaccines available for children and adolescents in Chile

Data from the Chilean government's pharmacovigilance indicate that CoronaVac, a vaccine from Butantan and Chinese pharmaceutical company Sinovac, is the immunizing agent with the lowest rates of adverse events in children and adolescents, aged between three and 17, who received Covid-19 vaccines in the country. CoronaVac is the safest immunizing agent both in general notifications and when compared to other vaccines and among people of different ages and sexes, according to data from the 4th Statistical Report "ESAVI associated with the administration of SARS-CoV-2 vaccines in Chile in the pediatric population and adolescents", from the local Ministry of Health.

Between March 1st, 2021 and February 26, 2022, 6.9 million doses of Covid-19 vaccines were administered to those under 18 years of age in Chile. Of these, 4.9 million doses were from CoronaVac and 2 million doses from Comirnaty, a Pfizer/BioNTech vaccine. In the same period, 868 Events Supposedly Attributable to Vaccination or Immunization (ESAVIs) were reported by Chilean pharmacovigilance among people aged three to 17 years who received both vaccines, with lower rates of adverse events among those who received CoronaVac.

ESAVI is an unfavorable, unintended health condition, which can be a symptom, a laboratory finding, or a disease, occurring after vaccination (vaccine administration) or immunization (generation of an immune response), according to the Pan American Health Organization (PAHO).

"The total number of notifications received associated with CoronaVac was 520, which corresponds to 0.01% of the total doses administered and a notification rate of 10.67 notifications per 100,000 doses," the report describes.

The messenger RNA vaccine (Pfizer), in turn, demonstrated a rate of 15.35 notifications of ESAVIs per 100,000 doses administered. The rate is higher than among those who took CoronaVac, even with a smaller number of doses administered in the analyzed age group.

These data prove that CoronaVac is a vaccine with rare adverse effects in children and adolescents and when they appear, they are usually limited to pain at the injection site in most reports. So much so that of the 520 notifications, 456 were of events considered non-severe, that is, symptoms disappear without the need for symptomatic treatment and hospitalization and that do not endanger life, as defined by PAHO.

The Chilean Ministry of Health authorized the use of CoronaVac in children aged 6 years and older on September 6, 2021 and in children aged 3 years and older on November 30, 2021. Messenger RNA vaccine has been used in the country in people aged 5 years and older since December 21, 2021.

The data were collected by the Pharmacovigilance Subdepartment (Subdepartamento de Farmacovigilancia, SDFV) of the Chilean Public Health Institute (Instituto de Salud Pública, ISP), linked to the local Ministry of Health, which notifies and investigates whether or not ESAVIs are related to vaccination.

CoronaVac sustains safety between doses

Notifications of adverse events between the first and second dose were also lower in those who took CoronaVac. According to the Chilean report, the first dose of CoronaVac was associated with 12.92 notifications and the second dose with 4.24 notifications of ESAVIs for every 100,000 doses administered. Among those who took the messenger RNA vaccine, there were 16.67 notifications of ESAVIs in the first dose and 10.41 in the second dose for every 100,000 doses administered.

Differences by age group and sex

Adverse event rates are also lower among boys and girls who have

taken CoronaVac compared to the other available vaccine. According to the report, the notification rate of ESAVIs among boys aged 12 to 17 who took CoronaVac is 9.45 notifications per 100,000 doses, while the rate in boys of the same age group who took the RNA vaccine messenger, is 14.57 for every 100,000 doses administered. Among girls aged 12 to 17 who took CoronaVac, the rate of ESAVIs was 10.58 per 100,000 doses, while among girls in the same age group who took the other vaccine, it was 15.46 notifications per 100,000 doses.

As for the age group from 3 to 11 years, adverse event data were computed only in relation to CoronaVac. Among boys in this age group, the rate was 10.76 notifications of ESAVIs for 100,000 doses and 11.06 notifications for 1,100 doses among girls in this age group.

Most common adverse effects

According to the document, pain at the injection site corresponds to 3.26 notifications for every 100,000 doses of CoronaVac, on average. Pruritus (1.99 notifications per 100,000 doses), headache (1.60 per 100,000 doses), vomiting (1.54 notifications per 100,000 doses), urticaria (1.42), fever (1.07) and nausea (1.05) are the other most frequent events. All data are fewer incidents than reported for messenger RNA vaccines.

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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

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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.

Article

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 años¹. 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.

Article

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 serias², 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.

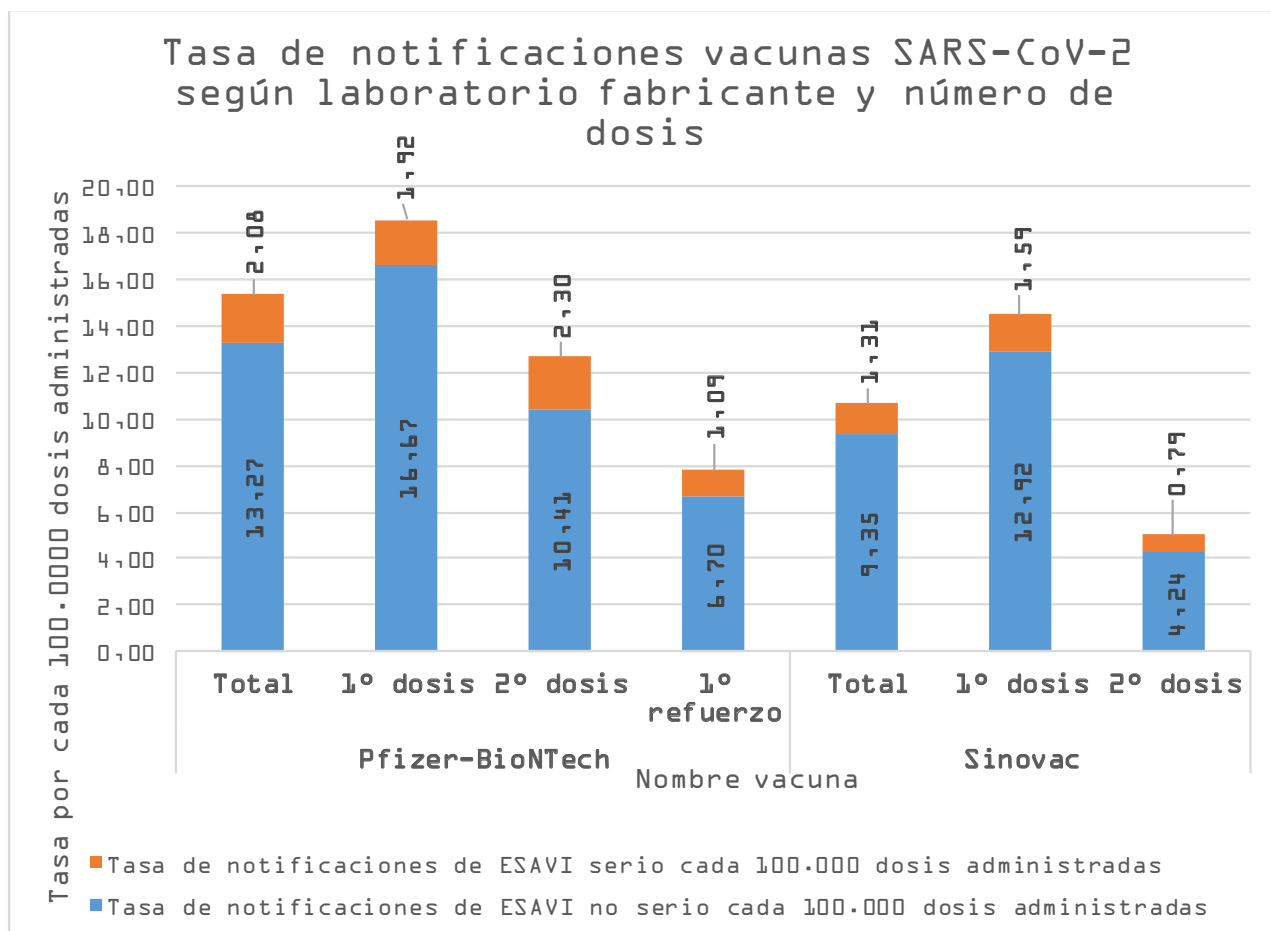


Figura 1. Tasa de notificaciones por 100.000 dosis administradas, según laboratorio fabricante y número de dosis administrada, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años.

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.

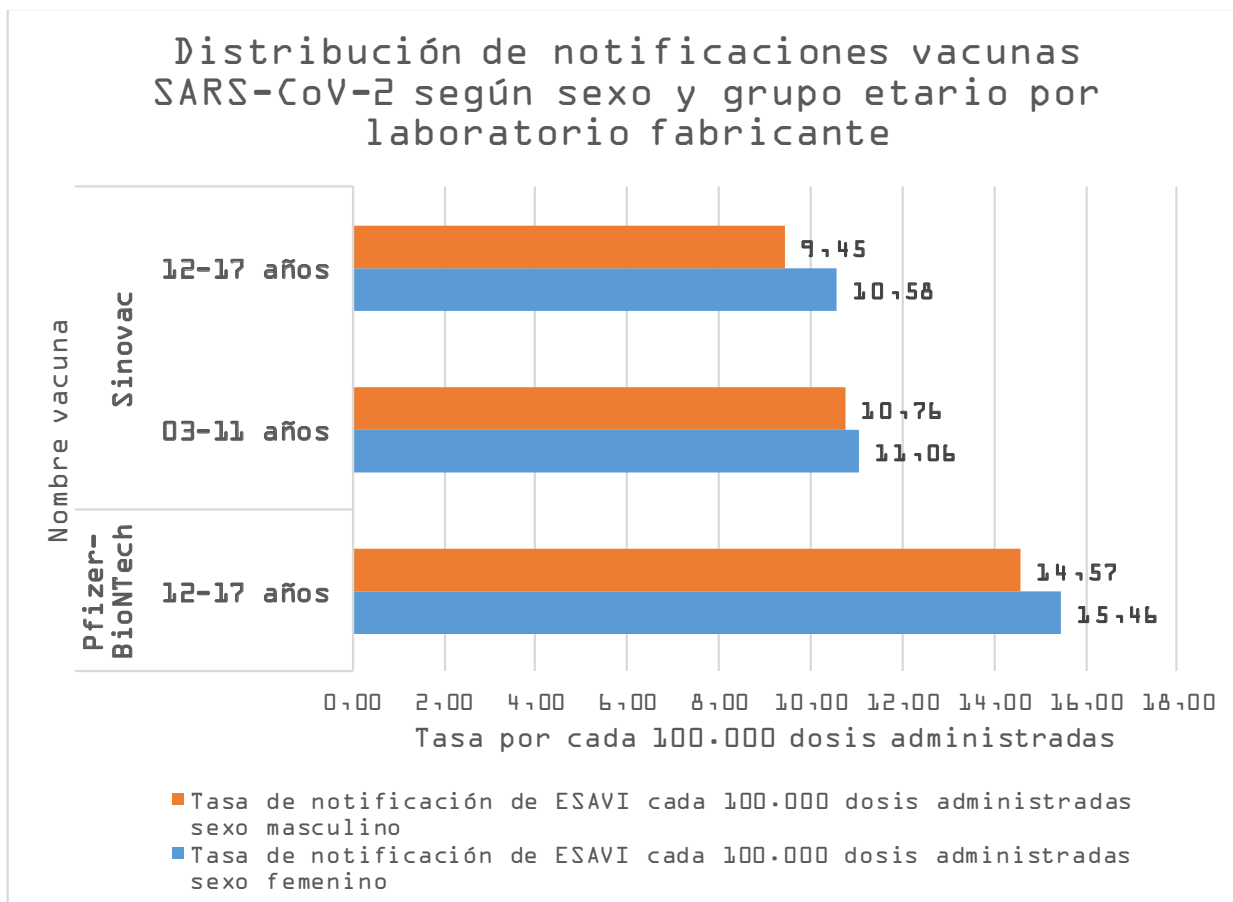


Figura 2. Distribución de notificaciones vacunas SARS CoV-2 según sexo y grupo etario, por laboratorio fabricante, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

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.

Article

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	Nº 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.

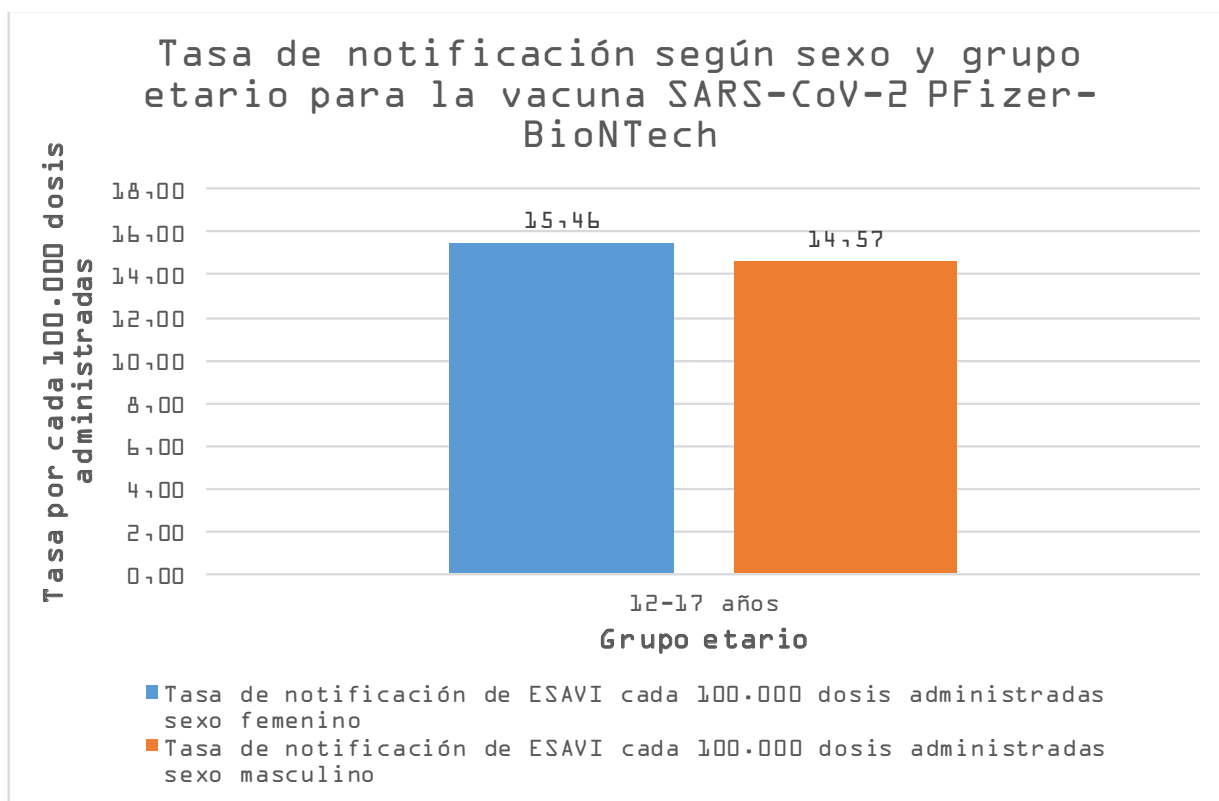


Figura 3. Tasa de notificación de ESAVI según sexo y grupo etario para la vacuna SARS-CoV-2 Pfizer-BioNTech, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

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.

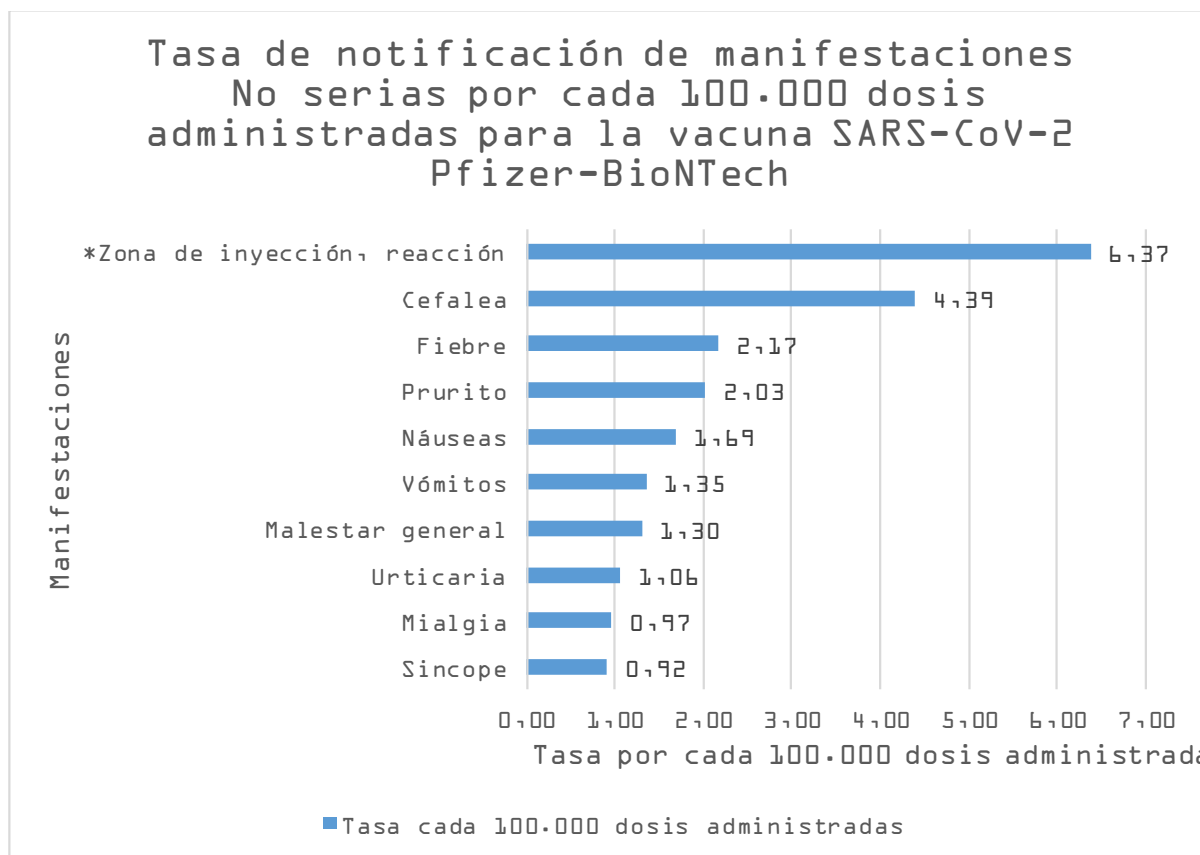


Figura 4. Tasa de notificación de manifestaciones clínicas No serias, más frecuentemente reportadas para la vacuna SARS-CoV-2 Pfizer-BioNTech, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

*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, hipertensión, nefritis e insuficiencia cardíaca. Estos no se informaron en el gráfico debido a que no se ubican dentro de las 10 primeras manifestaciones más frecuentes.

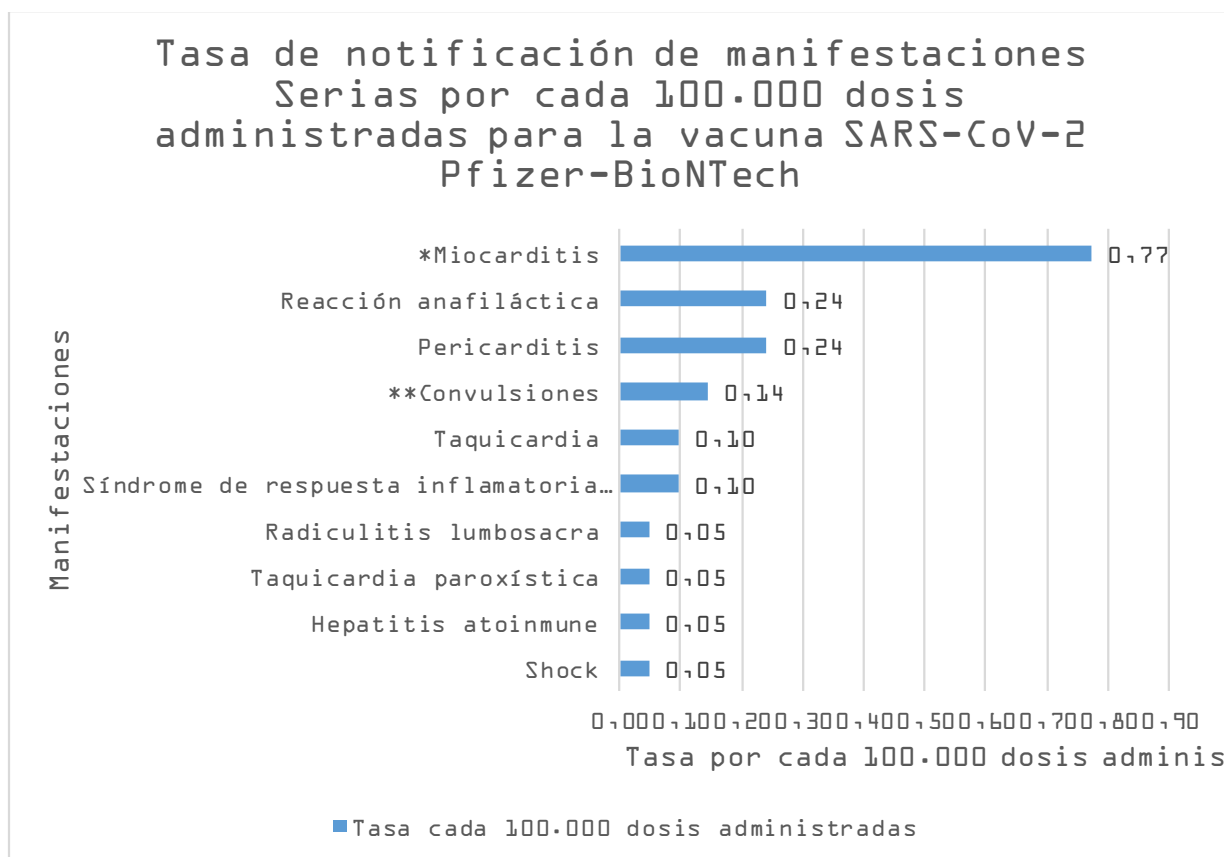


Figura 5. Tasa de notificación de manifestaciones clínicas serias más frecuentemente reportadas, para la vacuna SARS-CoV-2 Pfizer-BioNTech periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

*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.

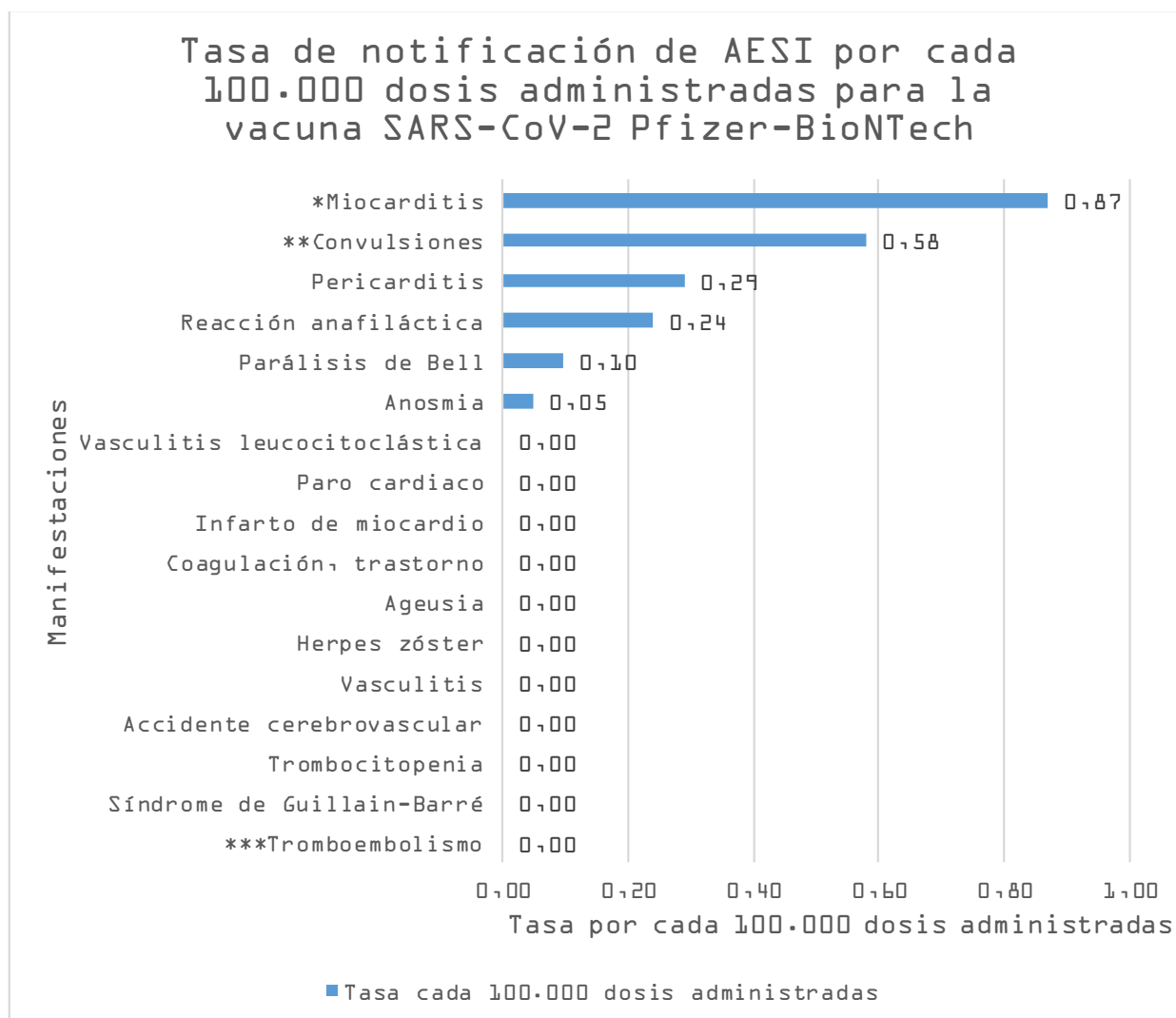


Figura 6. Tasa de notificación de manifestaciones clínicas de especial interés reportadas para la vacuna SARS-CoV-2 Pfizer-BioNTech, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

*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



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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, hipertensión 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

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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.

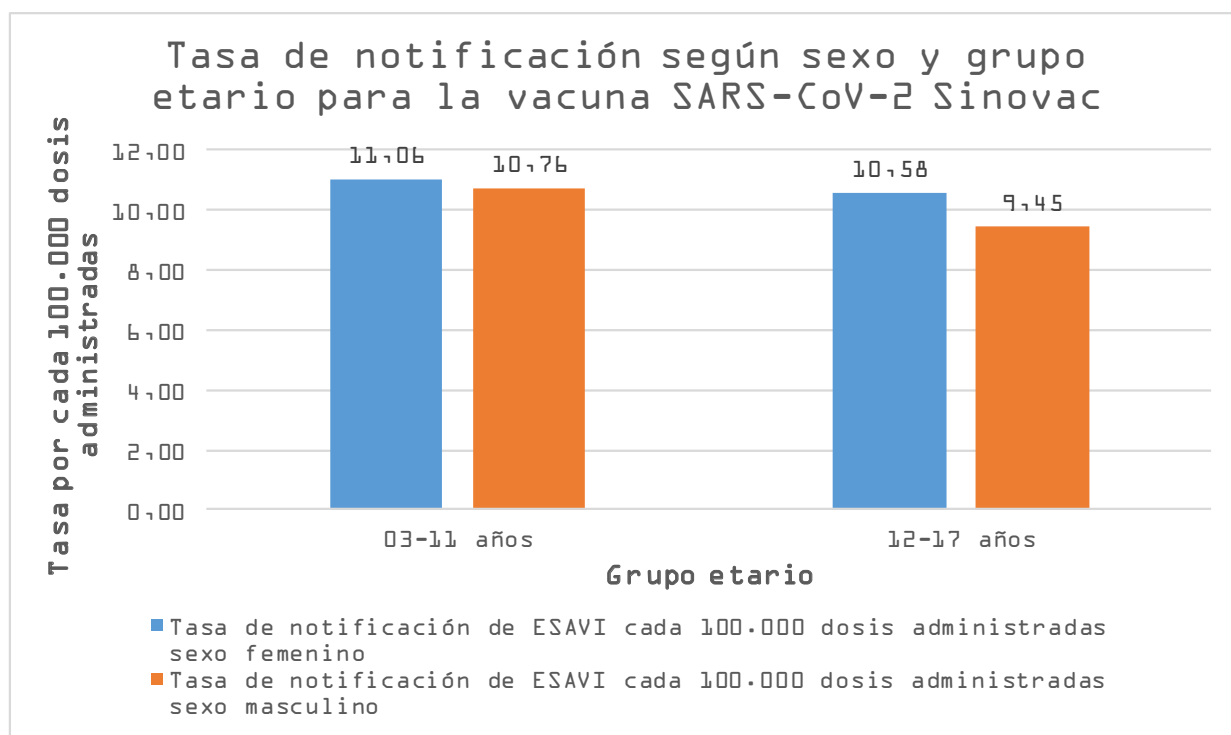


Figura 7. Tasa de notificación de ESAVI según sexo y grupo etario para la vacuna SARS-CoV-2 Sinovac, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

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.

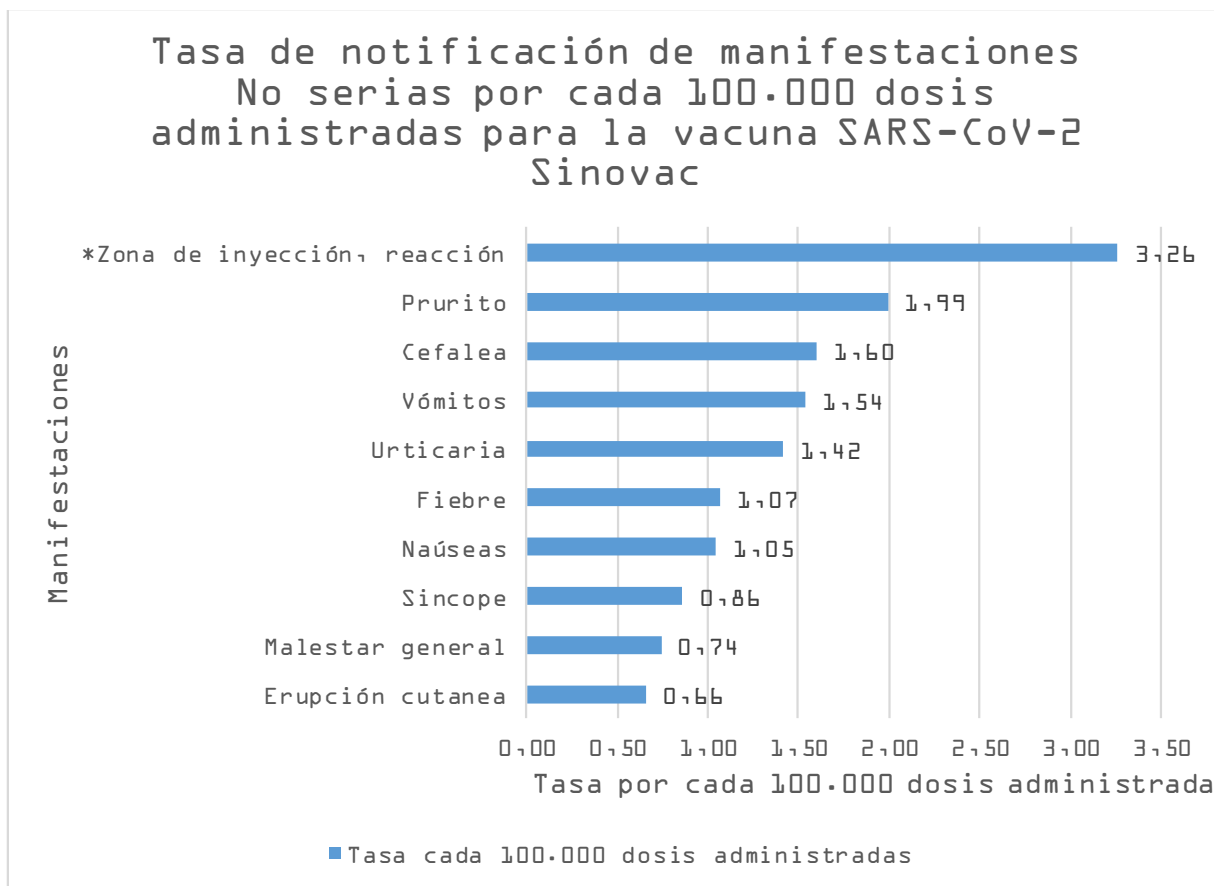


Figura 8. Tasa de notificación de manifestaciones clínicas no serias más frecuentemente reportadas para la vacuna SARS-CoV-2 Sinovac, periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

*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,

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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.

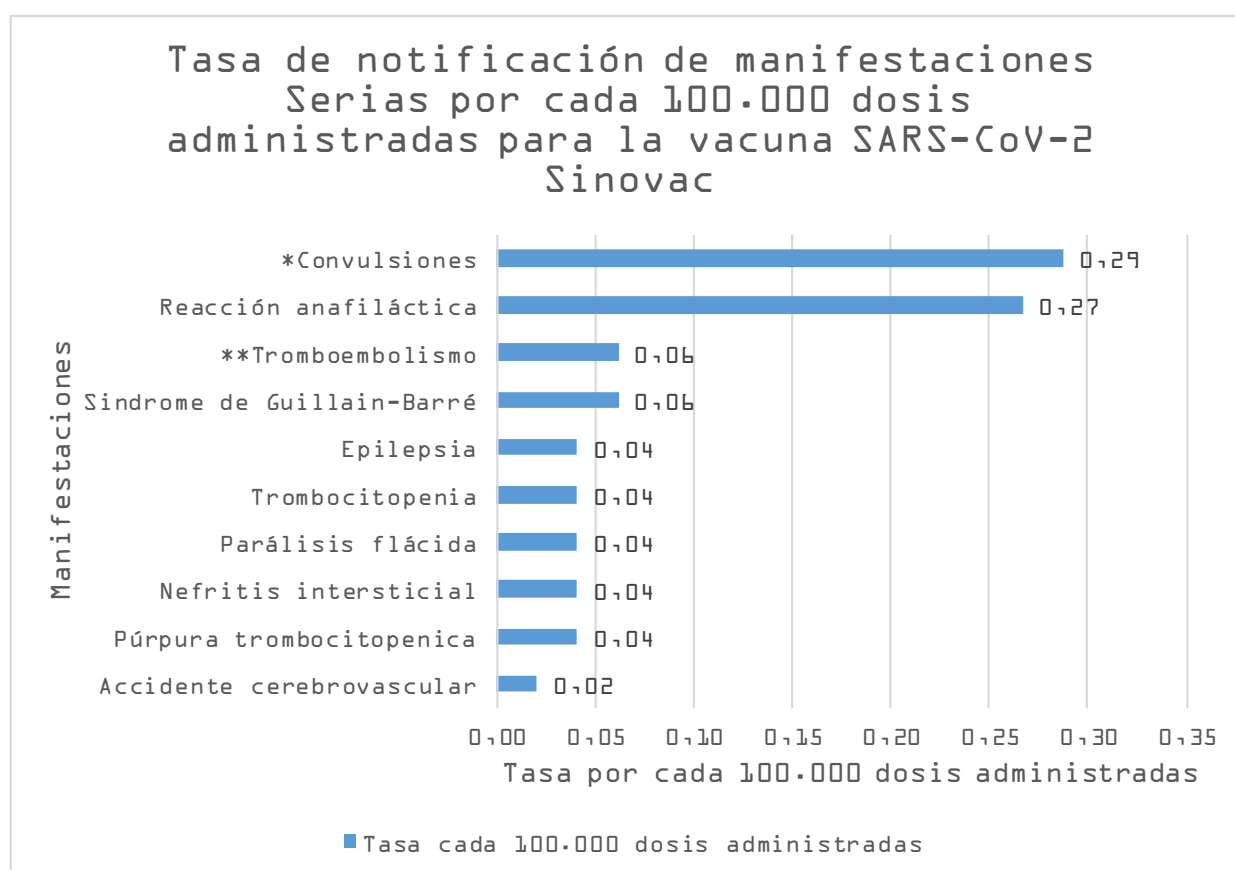


Figura 9. Tasa de notificación de manifestaciones clínicas serias más frecuentemente reportadas, para la vacuna SARS-CoV-2 Sinovac periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

*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.

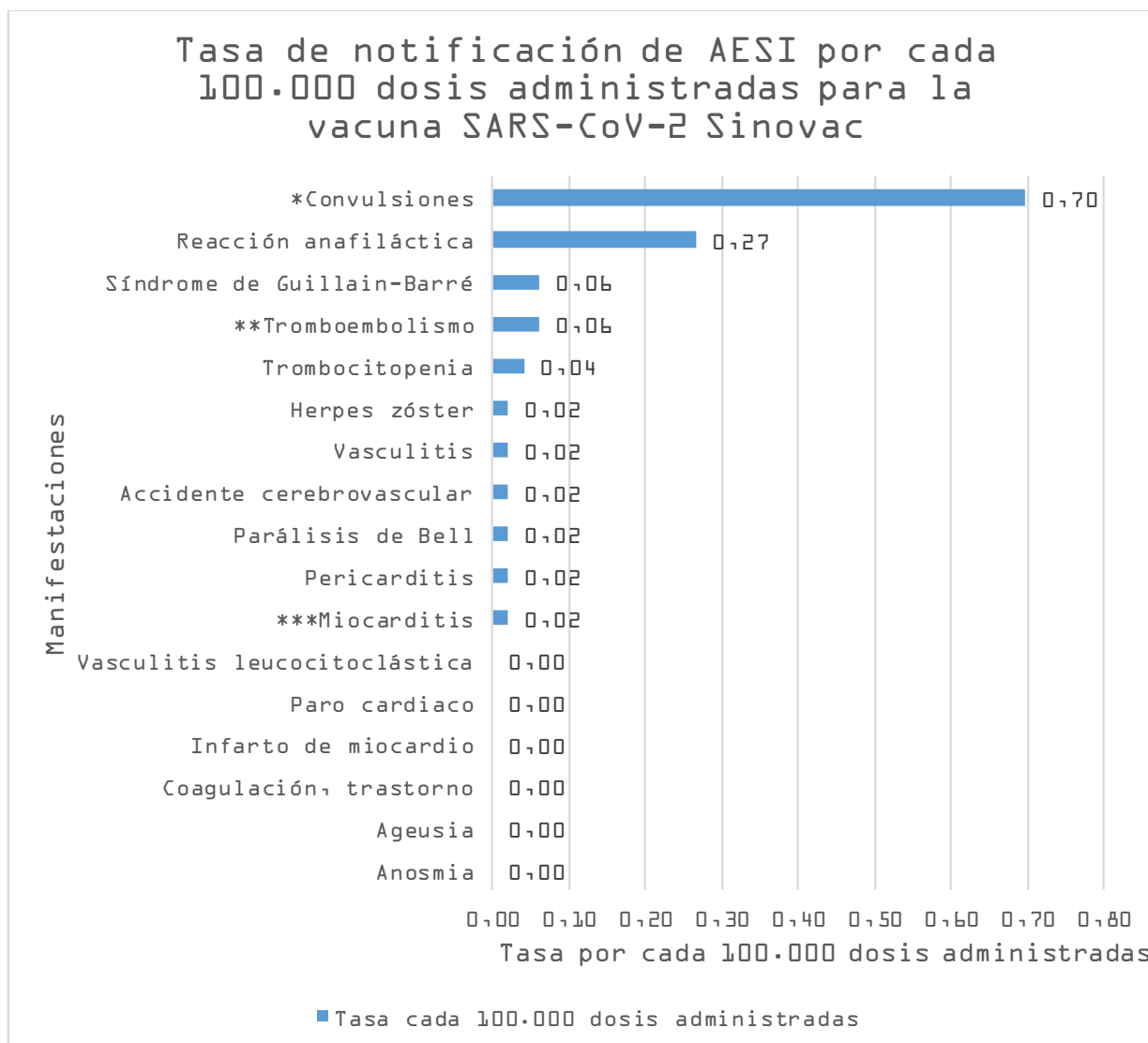


Figura 10. Tasa de notificación de manifestaciones clínicas de especial interés, reportadas para la vacuna SARS-CoV-2 Sinovac periodo 01 marzo de 2021 a 26 febrero de 2022, menores de 18 años

*convulsiones considera: convulsiones, convulsiones focales y convulsiones tónico/clónicas. **tromboembolismo considera: trombosis, Tromboembolismo y embolismo. ***miocarditis considera: miocarditis y miopericarditis

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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 <u>No serios</u>	% de ESAVI en la población vacunada	Tasa de notificación x 100.000 habitantes
761	0,01 %	10,96

ESAVI clasificados como <u>serios</u>	% 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.

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CoronaVac

What science proves

6.5. CoronaVac protected children as young as 3 during Shanghai omicron outbreak, Chinese study shows

A Chinese study conducted in March 2022, during the outbreak of the SARS-CoV-2 omicron variant in Shanghai, showed once again that CoronaVac is safe and protects children against Covid-19. Among infected children who showed symptoms, more than 70% had not yet been vaccinated, which reinforces the importance of immunization to prevent more serious outcomes. The article was published on the MedRxiv preprint platform and led by infectious disease specialists at Fudan University Children's Hospital in Shanghai.

A total of 376 children and adolescents aged up to 17 years (5 years on average) participated in the research, who were treated at the Chinese university hospital. Of this group, 250 had not yet been vaccinated, 110 had taken two doses of CoronaVac and 16 had received only one dose.

The analysis showed that, of the 257 symptomatic cases, 75% were unvaccinated children. Symptomatic infection was more frequent in the group aged less than 3 years (90/104), followed by the group aged 3 to 5 years (65/94) – only 5.3% of children in this age group had completed immunization. Among children aged 6 to 17 years, 59% were vaccinated, which provided greater protection for this public.

The experts' conclusion is that the high vaccine coverage against Covid-19, even during the omicron outbreak, reduced the risk of a serious infection in the pediatric public. "The mass COVID-19 vaccination rollout 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 the end of March in 2022."

They add that non-pharmacological interventions such as mask use indoors and hand hygiene, combined with vaccination strategies, are critical for preventing infection and severe disease and for mitigating SARS-CoV-2 transmission in the pediatric population.

The research results reaffirm the efficacy of using CoronaVac in children from 3 years of age, which had already been proven by a large study conducted in Chile. The work done with 500,000 children who took CoronaVac during the omicron outbreak showed that the Butantan and Sinovac vaccine is 69% effective against hospitalization in the Intensive Care Unit (ICU) and 64.6% against hospitalization due to Covid-19.

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1 **Epidemiological and clinical features of SARS-CoV-2 Infection in children during**
2 **the outbreak of Omicron Variant in Shanghai, March 7-March 31, 2022**

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26

27 **Abstract**

28 **Objectives:** To understand the epidemiological and clinical characteristics of pediatric SARS-CoV-2
29 infection during the early stage of Omicron variant outbreak in Shanghai.

30 **Methods:** This study included local COVID-19 cases <18 years in Shanghai referred to the exclusively
31 designated hospital by the end of March 2022 since emergence of Omicron epidemic. Clinical data,
32 epidemiological exposure and COVID-19 vaccination status were collected. Relative risks (RR) were
33 calculated to assess the effect of vaccination on symptomatic infection and febrile disease.

34 **Results:** A total of 376 pediatric cases of COVID-19 (median age:6.0±4.2 years) were referred to the
35 designated hospital during the period of March 7-31, including 257 (68.4%) symptomatic cases and 119
36 (31.6%) asymptomatic cases. Of the 307 (81.6%) children;3 years eligible for COVID-19 vaccination,
37 110 (40.4%) received 2-dose vaccines and 16 (4.0%) received 1-dose vaccine. The median interval
38 between 2-dose vaccination and infection was 3.5 (IQR: 3, 4.5) months (16 days-7 months). Two-dose
39 COVID-19 vaccination reduced the risks of symptomatic infection and febrile disease by 35% (RR 0.65,
40 95% CI:0.53-0.79) and 33% (RR 0.64, 95% CI: 0.51-0.81). Two hundred and sixteen (83.4%)
41 symptomatic cases had fever (mean duration: 1.7±1.0.8 days), 104 (40.2%) had cough, 16.4% had
42 transient leukopenia; 307 (81.6%) had an epidemiological exposure in household (69.1%), school (21.8%)
43 and residential area (8.8%).

44 **Conclusion:** The surge of pediatric COVID-19 cases and multiple transmission model reflect wide
45 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.

48 **Introduction**

49 The COVID-19 pandemic has caused devastation to the world's population, resulting in more than
50 6 million deaths as of April 20, 2022. SARS-CoV-2 infection in most pediatric cases is mild as compared
51 to adults and the direct effect on child health is limited [1]. However, the indirect impacts on child medical
52 care, education and mental health are considerable owing to lockdown, disruption of essential health
53 service delivery, prolonged school closure and isolation [2,3]. The continuous genetic evolution of
54 SARS-CoV-2 virus results in the emergence of multiple new variants of concern (VOC), which are
55 associated with enhanced transmissibility or increased virulence and immune escape [4]. The Omicron
56 variant, which was detected in November 2021 and almost replaced Delta variant by the end of January
57 2022, has led to the fifth global wave of COVID-19 epidemic [5]. The significant rise of pediatric
58 infection was reported in the United States with children aged <18 years, representing 17.0%-19.0% of
59 all cases during the Omicron period since late December 2021 [6,7].

60 Pediatric COVID-19 cases only accounted for a small proportion of infection in the early stages of
61 the COVID-19 pandemic when many countries implemented non-pharmaceutical interventions and strict
62 containment measures [8-12]. However, the incidence rate of COVID-19 in children showed a rising
63 trend in the epidemic countries following suspension of lockdown and school reopening [12,13]. After
64 the large-scale epidemic in early 2020, China entered a normalization stage of prevention and control,
65 and massive COVID-19 vaccination campaign was launched nationwide in 2021. Inactivated SARS-
66 CoV-2 vaccine BBIBP-CorV (by Sinopharm) and CoronaVac (by Sinovac) were approved for emergency
67 use in children 3-17 years on June 2021 and COVID-19 vaccination program was initiated in pediatric
68 population since late July 2021 across China. From May 2020, local pediatric COVID-19 infection linked
69 to sporadic and cluster transmission were occasionally reported in China until the community outbreak

70 of Omicron variant appeared in Hong Kong Special Administrative Region since January 6, 2022 and
71 subsequently in Shanghai since early March 2020 [14]. Omicron variant spread rapidly in Shanghai by
72 the end of March and led to a surge of pediatric COVID-19 cases citywide. Here, we describe
73 epidemiological and clinical characteristic of Omicron variant infections in Shanghainese children during
74 the early stage of the outbreak.

75

76 **Subject and Method**

77 **Subject**

78 In this study, we included local COVID-19 cases <18 years of age who were notified in Shanghai
79 and admitted to the exclusively designated hospital in Shanghai by the end of March 2022. Prior to 28
80 March, all pediatric COVID-19 cases notified in Shanghai were referred to the designated hospital for
81 concentrating management and isolation. Since 28 March when a large number of cases were confirmed
82 by massive screening test, most of asymptomatic and mild pediatric cases aged 5-17 years were almost
83 sent to Fangcang shelter hospitals. All confirmed cases irrespective of symptoms are required for in-
84 hospital isolation and are discharged if the cycle threshold (Ct) value for the viral nucleic acid is great
85 than 35 on PCR test for the two consecutive respiratory samples taken 24-hour apart [15].

86 **Case definition and classification**

87 All COVID19 cases were laboratory-confirmed by the Shanghai CDC reference laboratory using
88 real-time RT-PCR commercial kit. The Ct value <40 was defined as a positive nucleic acid amplification
89 test. COVID-19 cases were classified as asymptomatic and symptomatic cases. Symptomatic cases were
90 classified as mild, moderate and severe cases. An asymptomatic case is defined as a person with a positive
91 nucleic acid test but without any clinical symptom of COVID-19. A confirmed symptomatic case is

92 defined as a person presenting clinical signs and symptoms of COVID-19. COVID-19 disease severity
93 classification is based on the WHO guidance [16]. Pneumonia was diagnosed based on clinical signs
94 (fever and/or cough accompanying with one of the following signs: moist rales on auscultation, difficulty
95 breathing/dyspnoea, fast breathing, chest indrawing), radiological findings compatible with pneumonia.

96 **Data collection**

97 Data were collected via a face-to-face interview with parents or teenagers and electronic medical
98 chart, including: demographic information, epidemiological exposure setting, COVID-19 vaccination
99 status on dose and date, clinical symptoms, laboratory findings and chest imaging if examined, treatment
100 and outcome. Informed consent from parents was not required by the ethics committee because all data
101 were de-identified and not involved in personal privacy.

102 **Statistical analysis**

103 Data was entered into Excel version 2016 (Microsoft, Redmond, Washington) for analysis and the
104 statistical analysis was performed using SPSS (IBM Statistic 23.0). Categorical variables are described
105 as absolute numbers and percentage. Continuous variables with normal distribution are expressed as
106 mean \pm standard deviation. Median (25% to 75% interquartile range (IQR) are used when the frequency
107 distributions were skewed. Differences between groups are compared using Mann-Whitney *U*-test and
108 Student's *t*-test as appropriate. A difference with $P < 0.05$ is considered to be statistically significant.
109 Relative risks (RR) were calculated using proportion of symptomatic infection and febrile cases by
110 vaccination status, with the referent group being ≥ 2 -dose vaccinees.

111 **Results**

112 **Demographic characteristics**

113 The first local pediatric case was notified on March 2022 and increased remarkably from 14 March
114 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).

137 As shown in table 1, 2-dose COVID-19 vaccination reduced the risk of symptomatic infection and
138 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
139 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-
140 17 years eligible for COVID-19 vaccine. However, one-dose vaccination could not significantly decrease
141 the relative risks of symptomatic infection and febrile disease.

142 **Clinical manifestation and course**

143 Of the 376 cases, 257 (68.4%) presented symptoms and 119 (31.6%) had no symptoms before and
144 duration hospitalization. Of the 257 symptomatic cases, 216 (83.4%) experienced fever (axillary
145 temperature $>37.5^{\circ}\text{C}$) with a mean fever spike of $38.9 \pm 0.6^{\circ}\text{C}$ (range: $37.6\text{-}41^{\circ}\text{C}$) and a mean fever
146 duration of $1.7 \pm 1.0.8$ days (range: 0.5-4 days), 104 (40.2%) presented cough, 28 (10.8%) self-reported
147 sore throat, 13 (5.0%) self-reported stuffy nose, 6 (2.3%) had runny nose, 11 (4.2%) had nausea or
148 vomiting or diarrhea, 2 (0.8%) self-reported transient loss of taste and smell. No severe case was
149 diagnosed. Twenty five cases had chest CT performed due to fever $>38.5^{\circ}\text{C}$ lasting for 3 days or cough
150 worsening after admission or routine examination prior to the referral. The chest images showed patchy
151 infiltrates or ground-glass opacity in 4 cases and one of them was right lung lobar pneumonia caused by
152 *Mycoplasma pneumoniae*. Six (1.6%) cases had comorbidity including brain tumor, febrile seizure,
153 psychomotor retardation, hemophilia, Henoch-Schonlein purpura, and cardiac arrhythmia in each.

154 As shown in table 2, 22.8% (57/250) of unvaccinated cases were asymptomatic while 50.0%
155 (55/110) of 2-dose vaccinated cases were asymptomatic ($P=0.000$); 65.2% (163/250) of unvaccinated
156 cases were febrile while 41.8% (46/110) of 2-dose vaccinated cases were febrile ($P=0.000$).
157 Symptomatic infection was significantly frequently seen in the age group <3 years than in the age group
158 3-5 years ($P=0.003$) and 6-17 years ($P=0.000$). Fever was significantly frequently seen in the age group

159 3-5 years than in the age group <3 years (P=0.000) and 6-17 years (P=0.005).

160 Of the 225 case who had complete peripheral blood cell count tested, 37 (16.4%) had white blood
161 cell (WBC) count $<4 \times 10^9/L$, 173 (76.9%) had WBC count $4-9 \times 10^9/L$, 13 (5.8%) had WBC count $10-14 \times 10^9/L$ and 2 (7.1%) had WBC count $\geq 15 \times 10^9/L$. The WBC count ranged from $1.9 \times 10^9/L$ to
162 $15.5 \times 10^9/L$. No thrombopenia was observed. Of the 187 cases who had peripheral blood C-reactive
163 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)
164 and 1 (0.5%) had CRP 56 mg/L who had co-infection with *mycoplasma pneumoniae* and developed
165 typical lobar pneumonia in right lung. Of the 196 cases who had serum biochemical markers and 8 (4.1%)
166 showed slightly elevated liver enzyme.

168 For symptomatic cases, Ibuprofen and or Chinese traditional medicines were prescribed depending
169 on the personalized condition and medication compliance. Only one case who had a clear diagnosis of
170 mycoplasma pneumonia was prescribed antibiotics. All cases were discharged when the Ct value of the
171 nucleic acid of SARS-CoV-2 virus reached >35 . The average duration of Ct value of the nucleic acid of
172 SARS-CoV-2 virus >35 since admission was 11.7 ± 3.7 days (range: 3-25 days; symptomatic verse
173 asymptomatic: 11.7 ± 3.6 verse 11.7 ± 3.9 , P=0.064).

174

175 Discussion

176 This study first presents the epidemiological and clinical profiles of Omicron variant infection in
177 localized children during the early phase of outbreak in Shanghai. As of 31 March 2022, all pediatric
178 COVID-19 cases were mild (68.4%) or asymptomatic (31.6%). However, a few of severe pediatric cases
179 were reported during the period of COVID-19 outbreak in Wuhan in early 2020 [17]. Moreover, the
180 proportion of asymptomatic cases was 2-time more than that seen in the Wuhan outbreak. We reason that

181 high coverage of COVID-19 vaccination among Shanghainese children is very likely to lower the risk of
182 severe Omicron infection-associated disease. The mass COVID-19 vaccination roll-out among children
183 3-17 years started between Mid-Aug 2021 and December 2021 in Shanghai and the estimated coverage
184 rate of 2-dose COVID-19 vaccination among children 3-17 years has exceeded 70% by the end of
185 March in 2022. In this case cohort 46.3% of children eligible for COVID-19 vaccination prior to 16 days
186 to 7 months (median: 3.5months). Observational studies from some countries with the high levels of
187 population immunity generated by natural infection or vaccine have shown receipt of two doses of
188 COVID-19 vaccines and a booster dose can offer protection against symptomatic and severe Omicron
189 infection in a short-term period of vaccination [4,18-22].

190 Current evidences consistently show a reduction in neutralizing antibody against Omicron in serum
191 of convalescent or vaccinated individuals, resulting in Omicron's immune escape potential against
192 vaccine- and infection-induced immunity [4,23]. However, two recent study based on real-world
193 observation among children showed the modest effectiveness for COVID-19 vaccine against Omicron
194 infection [25,26]. Based on our findings, receipt of 2-dose inactivated COVID-19 vaccine within 17 days
195 to 7 months after fully primary vaccination potentially reduced the risk of symptomatic Omicron
196 infection by 31% and febrile disease by 59% in children. We did not estimate vaccine protection against
197 severe infection because no severe COVID-19 cases were diagnosed. There is also evidence of waning
198 of vaccine effectiveness over time of the primary series against infection and symptomatic disease for
199 the studied vaccines. However, the vaccine effectiveness against Omicron infection and disease can be
200 restored and increase to > 40% to 80% within a short follow-up time after a third booster dose in studies
201 from five countries (United Kingdom, Denmark, Canada, South Africa, USA) [4]. Ten cases of
202 reinfection with Omicron variant were identified within 23 to 87 days of a previous Delta infection was

203 reported in the USA and most were pediatric cases [26]. Thus, eligible children and adolescents should
204 remain up to date with recommended COVID-19 vaccination in response to Omicron outbreak. So far, a
205 third booster dose of COVID-19 vaccine has been recommended for use in adults but not in children in
206 China. In light of the field findings, a booster dose should also be recommended for eligible children 3-
207 17 years.

208 We observed that school children aged 6-11 years comprised the most cases, followed by home-care
209 children <2 years and preschool children 3-5 year. The distribution of age groups in the early stage of
210 outbreak reflects the cluster transmission of COVID-19 centered in elementary school, kindergarten, and
211 household. Of note, age group 12-17 years accounted for the smallest proportion of pediatric cases,
212 among which, the high coverage rate of COVID-19 vaccination was as high as 95%. Based on the
213 epidemiological investigation, more than 80% children had a clear history of exposure, mostly occurring
214 in family (69.1%) and school (21.8%), occasionally in residential area (8.8%). The remaining 18.4% of
215 children had no clear contact with confirmed cases, reflecting small-scale community transmission had
216 already appeared prior to the large-scale outbreak since April. During the 2020 outbreak of COVID-19,
217 80%-90% of confirmed child cases were family cluster cases and community transmission was unusual
218 in China [17,27]. Rapid increases in pediatric COVID-19 cases and epidemiological unrelated cases also
219 suggest the occurrence of high community transmission of Omicron variant in Shanghai since the early
220 epidemic wave.

221 We observed most of localized pediatric cases (83.4%) of symptomatic Omicron infection
222 presenting fever. However, fever is less commonly seen in pediatric COVID-19 cases reported in China
223 (58%) and the USA (56%) during the first wave of pandemic in 2020 [10, 17]. Fever could be helpful for
224 early recognition and diagnosis of COVID-19 because parents always worry about the febrile child and

225 visit hospital for seeking medical care. The febrile course of Omicron infection is brief with a mean fever
226 duration of $1.7\pm 1.0.8$ days, significantly shorter than fever duration seen influenza (4 days) [28]. The
227 febrile duration is helpful to differentiate COVID-19 from influenza in children when the epidemics of
228 COVID-19 and influenza overlap.

229 The potential role in transmission for most asymptomatic and mild child cases should not be
230 neglected. A study showed that symptomatic and asymptomatic children can carry high quantities of live
231 SARS-CoV-2, creating a potential reservoir for transmission [29]. Vaccinees with mild or asymptomatic
232 Omicron infection shed infectious virus 6-9 days after onset or diagnosis, even after symptom resolution
233 [30]. In fact, asymptomatic infection in children was underestimated in the early stage of outbreak
234 because massive screening of COVID-19 cases had not been carried out before 28 March. After citywide
235 large-scale screening, notifiable asymptomatic cases accounted for 90% more or less in April.
236 Asymptomatic infection was much more common in vaccinated children than in unvaccinated children
237 (50% vs 22.8%). Vaccination can offer protection against symptomatic infection and febrile disease, on
238 the other hand, the role of asymptomatic children play in viral transmission is of attention during outbreak.
239 High prevalence of asymptomatic infection is likely a major factor in the widespread of the Omicron
240 variant among population.

241 In summary, COVID-19 is mild and subtle in Shanghainese children with the high level of vaccine-
242 induced immunity during the early stage of Omicron outbreak. COVID-19 vaccination can offer partial
243 protection against symptomatic COVID-19. Ongoing Omicron epidemic will increase the risk of
244 exposure among children with underlying medical conditions, who are usually unvaccinated, therefore,
245 severe COVID-19 infection is anticipated to be encountered in children. Non-pharmaceutical
246 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
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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-
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264

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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

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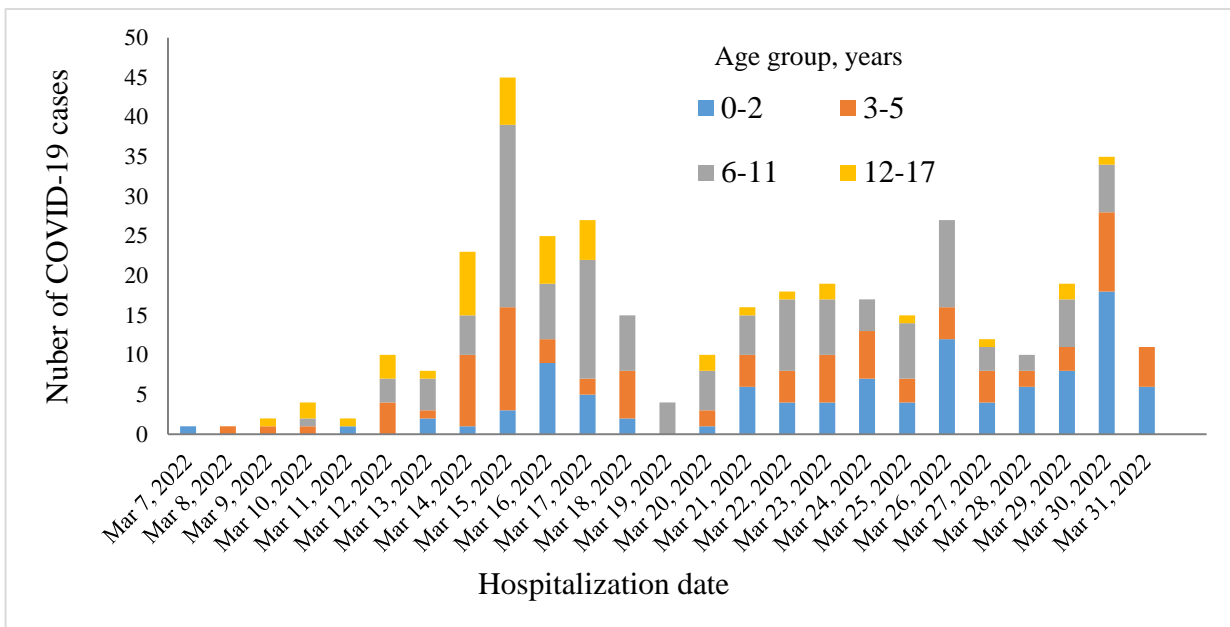


Figure 1. Daily COVID-19 cases referred to the designated hospital for children aged <18 years from March 1 to March 31, 2022

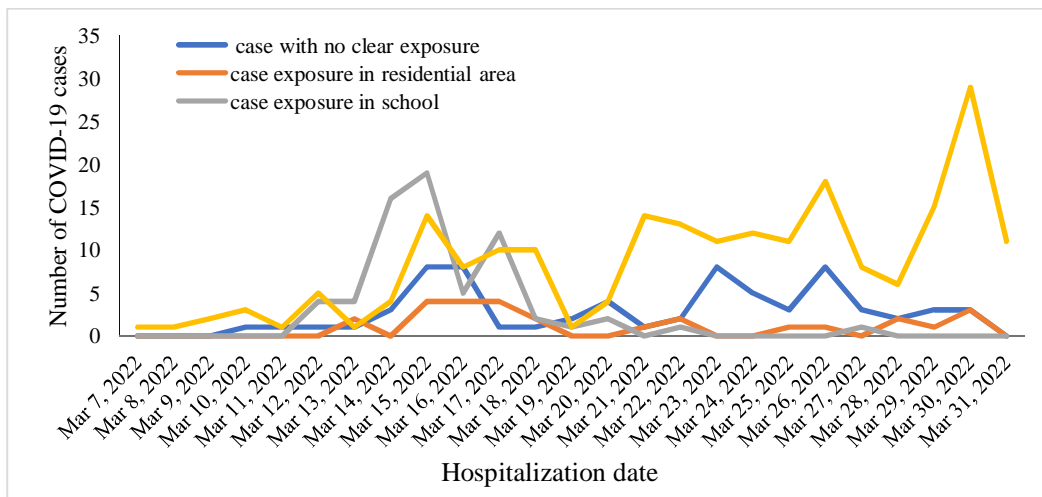


Figure 2. Model of epidemiological exposure over time among pediatric COVID-19 cases



CoronaVac

What science proves

6.6. CoronaVac is 69% effective against hospital admission for Covid-19 of children aged 3 to 5 years, says Chilean study carried out during the omicron outbreak

The work done with 500,000 children who took CoronaVac during the omicron outbreak in Chile showed that the Butantan and Sinovac vaccine is 69% effective against admission in the Intensive Care Unit (ICU), 64.6% against hospitalization due to Covid-19 and 38.2% against infection.

The study was published on the Research Square preprint platform and still needs peer review.

Evidence against omicron

The study group included 516,250 children aged three to five years affiliated with the National Health Fund (FONASA), Chile's public health system. Of these, 490,694 received CoronaVac and the others in the control group did not receive the vaccine. Children who tested positive for Covid-19 were excluded from the study.

The children took two doses of CoronaVac, 28 days apart, between Dec. 6, 2021 and Feb. 26, 2022, during the country's Covid-19 immunization campaign. According to the research, "our estimates provide evidence of vaccination effectiveness in children aged 3 to 5 years during the omicron outbreak in Chile."

The researchers recall recent preliminary estimates of the efficacy of vaccinating two doses of CoronaVac in children aged six to

16 years, at a time when delta was the predominant circulating variant of SARS-CoV-2.

In the previous study, the estimated efficacy of CoronaVac was 74.5% for preventing Covid-19, 91% for preventing hospitalization, and 93.8% for preventing Covid-19-related ICU admission. The estimates for the subgroup of children aged six to 11 years were 75.8% for prevention against Covid-19 and 77.9% for prevention of hospitalization for the disease.

"While the estimates are not directly comparable, the lower estimated vaccine effectiveness could be due to omicron or because the cohort included younger children," the researchers describe.

They reiterate that recent research suggests that vaccines may be less effective against omicron and that observational studies have limitations. "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," the study described.

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Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Effectiveness of CoronaVac in children 3 to 5 years during the omicron SARS-CoV-2 outbreak

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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 1000 person-days	Weighted, standard adjustment †	Weighted, stratified 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 York¹³ 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,

$i = 1, \dots, n$. Let \mathbf{x}_i , $i = 1, \dots, n$, 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

$$T_i | \mathbf{x}_i, z_i \stackrel{ind.}{\sim} f(t | \mathbf{x}_i, z_i), \quad i = 1, \dots, n,$$

where

$$f(t | \mathbf{x}_i, z_i) = \lambda_0(t) \exp \{ \mathbf{x}'_i \boldsymbol{\gamma} + \beta_{z_i(t)} \} \times \exp \left\{ - \exp \{ \mathbf{x}'_i \boldsymbol{\gamma} + \beta_{z_i(t)} \} \int_0^t \lambda_0(u) du \right\},$$

with $\boldsymbol{\gamma} \in \mathbb{R}^p$ being a vector of regression coefficients, $\beta_k \in \mathbb{R}$ being the regression coefficient measuring the effectiveness of the k^{th} vaccine, and λ_0 being the baseline hazard function

$$\lambda_0(t) = \lim_{h \rightarrow 0} \left\{ \frac{P_0(t \leq T \leq t + h | T \geq t)}{h} \right\},$$

where 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

$$f(t | \mathbf{x}_i, z_i) = \lambda_{\mathbf{x}_i, 0}(t) \exp \{ \beta_{z_i(t)} \} \times \exp \left\{ - \exp \{ \beta_{z_i(t)} \} \int_0^t \lambda_{\mathbf{x}_i, 0}(u) du \right\},$$

with $\beta_k \in \mathbb{R}$ being the regression coefficient measuring the effectiveness of the k th vaccine, and $\lambda_{\mathbf{x}, 0}$ 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 $100\% \cdot (1 - \exp\{\beta_k\})$. 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 package²⁵ 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

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Supplementary Files

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- [MSCovid35SuppMatNMedv10.docx](#)
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6.7. CoronaVac shows more than 90% effectiveness against Covid-19 in children, large Chilean study indicates

Last Tuesday (15), a study conducted in Chile showed that the effectiveness of CoronaVac in children and adolescents aged six to 16 years reached more than 90% against hospitalizations and admissions related to Covid-19, reinforcing the importance of immunizing this group. This is the first effectiveness study of CoronaVac performed in children, that is, it evaluates the efficacy of the immunizing agent with real-world data. It was conducted by the Chilean Ministry of Health, the Pontifical Catholic University of Chile and Harvard University in the United States, among other institutions, and was published on The Lancet's SSRN preprint platform.

"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," the authors point out.

The scientists included two million children and adolescents aged six to 16 years in the research, divided into two groups: immunized with two doses

of CoronaVac and not vaccinated. In the total cohort group, 12,735 Covid-19 infections, 207 hospitalizations and 30 ICU admissions associated with the coronavirus were observed.

Results

In children and adolescents aged between six and 16 years who took the vaccine, the effectiveness of CoronaVac was 74.5% to prevent infection, 91% against hospitalizations and 93.8% to avoid Intensive Care Unit (ICU) admissions. The effectiveness of the immunizing agent against death was not estimated, as no deaths were reported in this age group during the study period.

In a subgroup of children aged six to 11 years, the effectiveness was of 75.8% against the disease and 77.9% to prevent hospitalizations. No vaccinated children were admitted to the ICU, but six children who did not take the immunizing agent required hospitalization. The lower numbers in relation to the 6-16 group can be explained by the few hospitalizations in this population during the study period.

In the analyses, the researchers considered socioeconomic and demographic factors and included individuals with comorbidities such as chronic kidney disease, diabetes, cancer, cardiovascular disease, HIV, epilepsy, hemophilia, asthma, cystic fibrosis, juvenile idiopathic arthritis, and lupus.

“Our results are consistent compared to the CoronaVac effectiveness study in Chilean individuals over 16 years of age, previously published in 2021, which attested to 95% efficacy in preventing infections, 87.5% against hospitalizations, and 90.3% for ICU admissions in adults,” the scientists state.

Vaccination saves children’s lives and stops coronavirus transmission

Chilean researchers draw attention to the many benefits of immunizing children, such as preventing severe cases and death in this population and avoiding other complications of Covid-19, such as Multisystem Inflammatory Syndrome in Children (MIS-C), a severe inflammatory

response of the body that can lead to death.

“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,” they point out.

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Effectiveness of an inactivated SARS-CoV-2 vaccine in children and adolescents: A large-scale observational study

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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.

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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 an 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.

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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.

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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*

Characteristic	No.	Col.%	COVID-19		Unvaccinated		Vaccinated					
			No.	Row%	No.	Row%	One dose		Two doses		Three doses	
			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 ($p < 0.001$) 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	Person-days	Cases		Vaccine effectiveness (95% CI)	
		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	Person-days	Cases		Vaccine effectiveness (95% CI)	
		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.) (≥14 days after 2 dose)	78,449,194	1,502	0.0191	75.8 (74.8–76.8)	75.8 (74.7–76.8)
Hospitalization					
Unvaccinated	155,434,360	61	0.0004	–	–
CoronaVac (6-11 yr.) (≥14 days after 2 dose)	78,940,292	8	0.0001	78.5 (62.8–87.6)	77.9 (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.

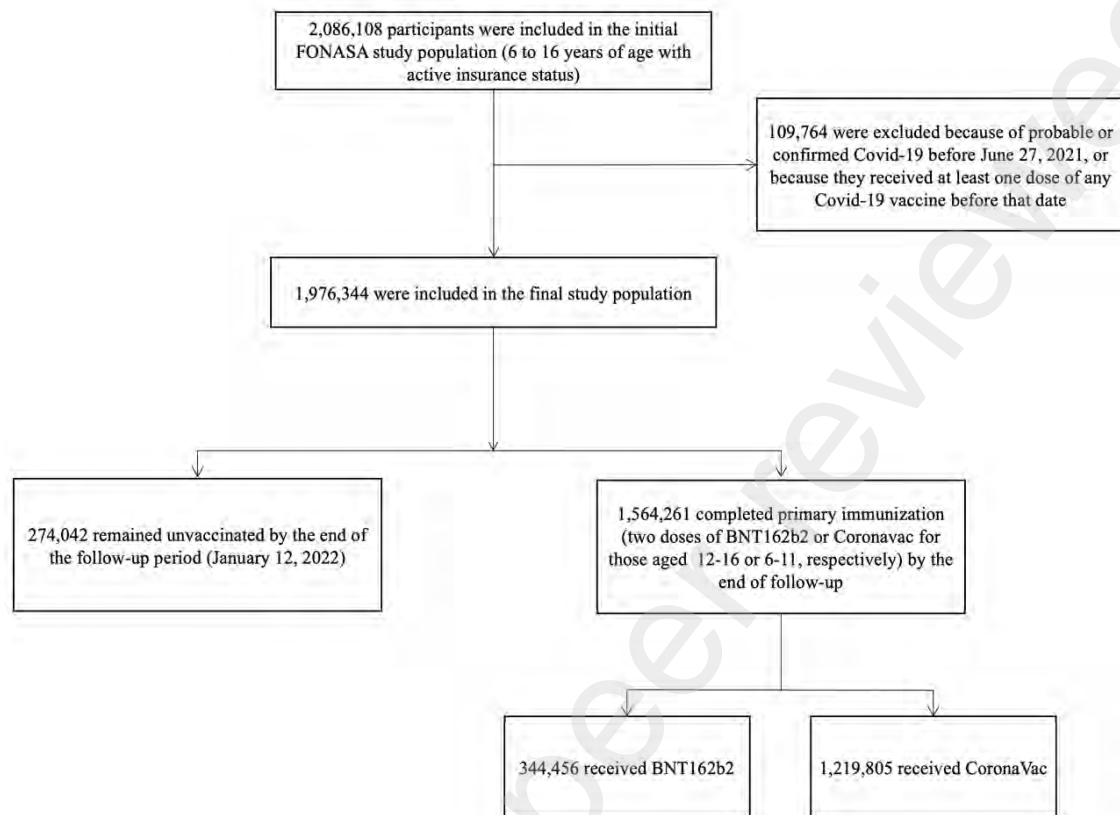


Figure 1. Study participants and cohort eligibility, June 27, 2021, to January 12, 2022. Participants were between 6 to 16 years of age, affiliated to the Fondo Nacional de Salud (FONASA), the public national healthcare system, and vaccinated with a complete primary immunization (2 doses 28 days apart) 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

What science proves

6.8. During the outbreak of Delta in China, around 20% of the cases were in children and teenagers; vaccinated with CoronaVac did not register critical cases

In a study published in January of 2022 in the PLOS Neglected Tropical Disease journal, Chinese researchers from the Southern Medical University of Guangzhou (Canton) and the Center for Disease Control and Prevention of the province pointed out that one in five cases from the outbreak of the delta variant of SARS-CoV-2, that happened in the region between May and June of 2021, affected minors with preschool age (1 to 5 years old) and students from 6 to 18 years of age. Besides, from the 153 cases of Covid-19 in the outbreak, around 85% happened among the non vaccinated.

During the period of the study, there were seven asymptomatic and 146 symptomatic cases. From those, 24 (15,7%) were considered mild, 113 (73,9%) moderate, and nine (5,9%) were considered critical. There were no severe cases. From the 153 cases, 116 (84,7%) happened in non vaccinated individuals and 21 (15,3%) on people with partial or complete vaccine scheme with CoronaVac, immunizer from Butantan and the Chinese pharmaceutical Sinovac, or Sinopharm, Chinese vaccine that is also made with inactivated virus. There were 16 excluded cases with undetermined vaccinal status.

“The clinical symptoms were lighter in the cases with partial or complete vaccination than in those that

were not vaccinated. Notably, no critical case was observed on those that were partially or completely vaccinated, while the nine critical cases happened all among unvaccinated people”, emphasized the researchers in the study.

From the total number of Covid-19 cases of the outbreak, 28 (18,3%) were among minors of 18 years of age, 72 (47,1%) among people from 19 to 59 years of age, 19 (12,4%) in the population from 60 to 70 years old and 34 (22,2%) on elderly above 70 years of age. Children in preschool age were 3,3% of the cases.

Intensification of vaccination after the outbreak

On 21st May of 2021, it was reported the first case of the delta variant in Guangzhou. As a response to the ressurging of Covid-19 in the province, the local government implemented a series of measures of containment and began the emergency vaccination of the whole population. By the end of June, when the outbreak was over, 10,7 million from the 15,3 million habitants were vaccinated with CoronaVac or Sinopharm (being 8,7 million that completed the vaccinal scheme of two doses), extending the vaccine coverage to 67% of the population of the province.

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RESEARCH ARTICLE

Transmission and containment of the SARS-CoV-2 Delta variant of concern in Guangzhou, China: A population-based study

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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]).

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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>).

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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 (infecter). 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 ≥ 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

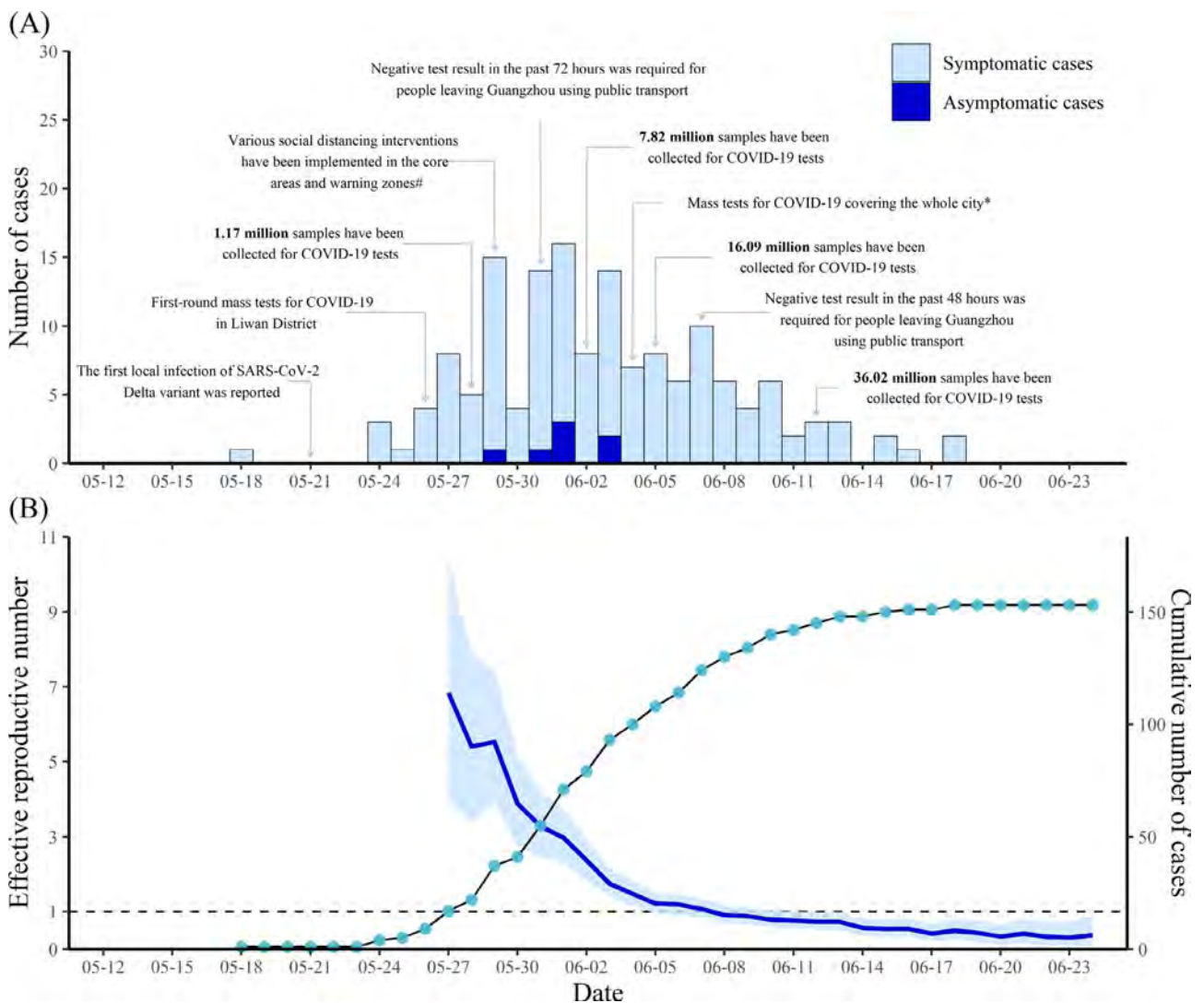


Fig 1. Number of COVID-19 cases by date of illness onset and effective reproductive number in Guangzhou, China. (A) Number of COVID-19 cases by date of illness onset. (B) Estimated effective reproductive number by ending date of 7-day time window and cumulative number of cases by date of illness onset. 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.

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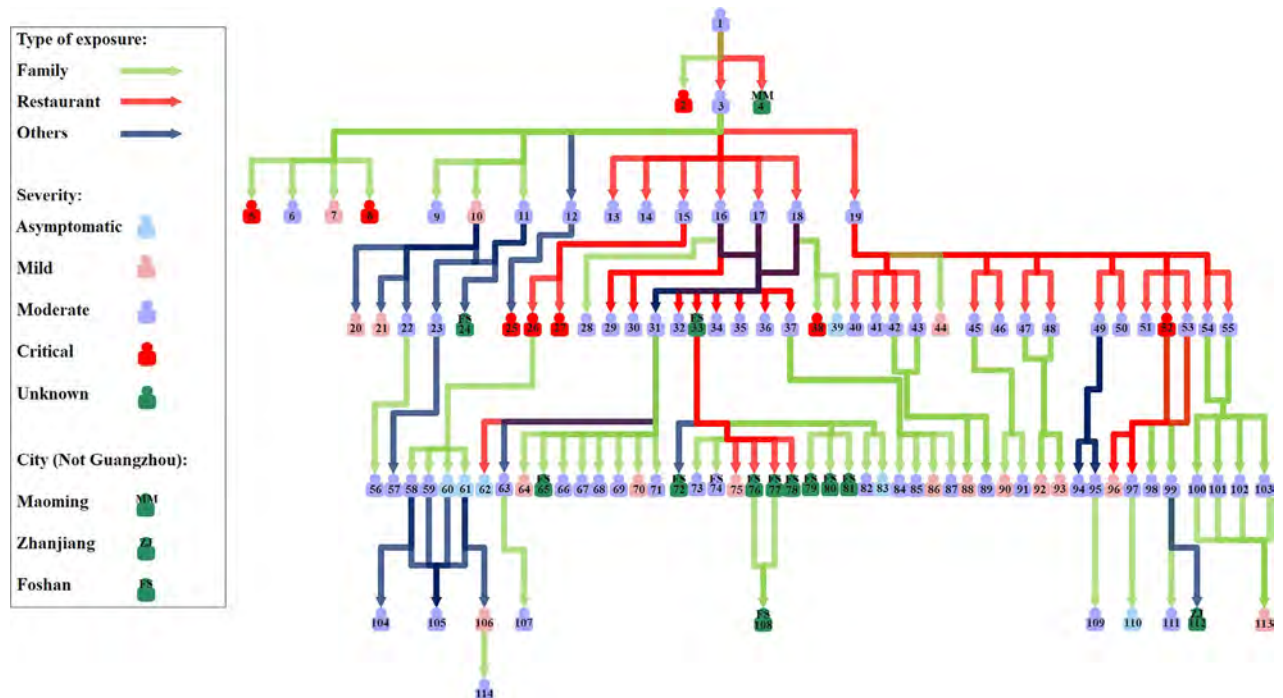


Fig 2. Transmission network of the infections of the SARS-CoV-2 Delta variant. A total of 101 and 13 cases reported in Guangzhou and other cities with information for determining the generation are presented. Cases without a clear epidemiological link with the confirmed cases and the ones whose infector did not have a clear exposure history were not included.

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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)

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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

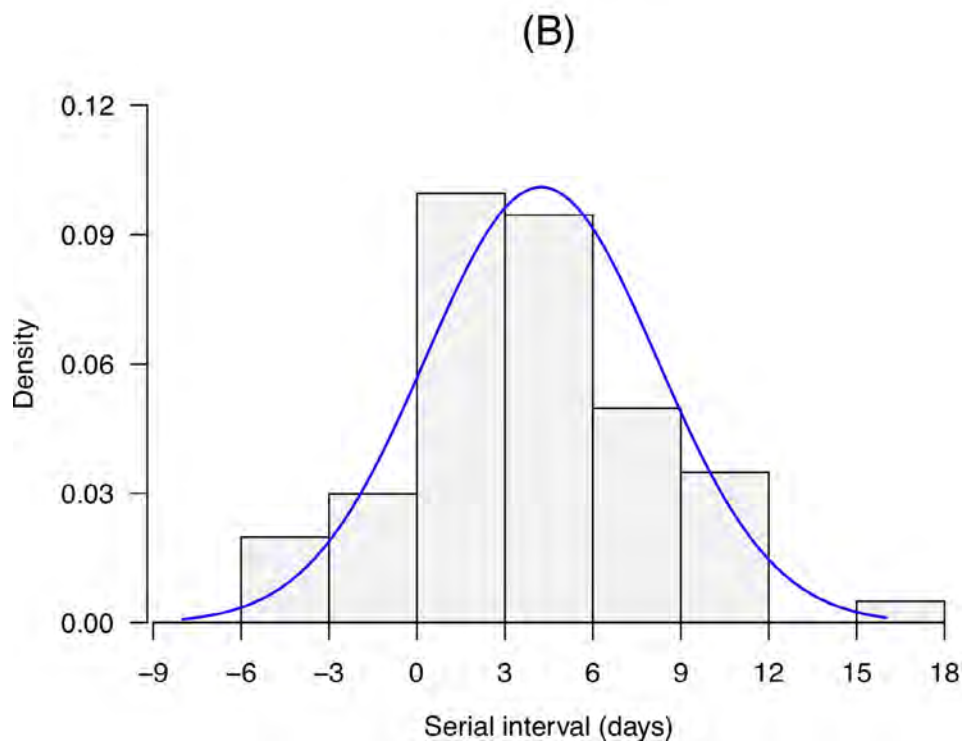
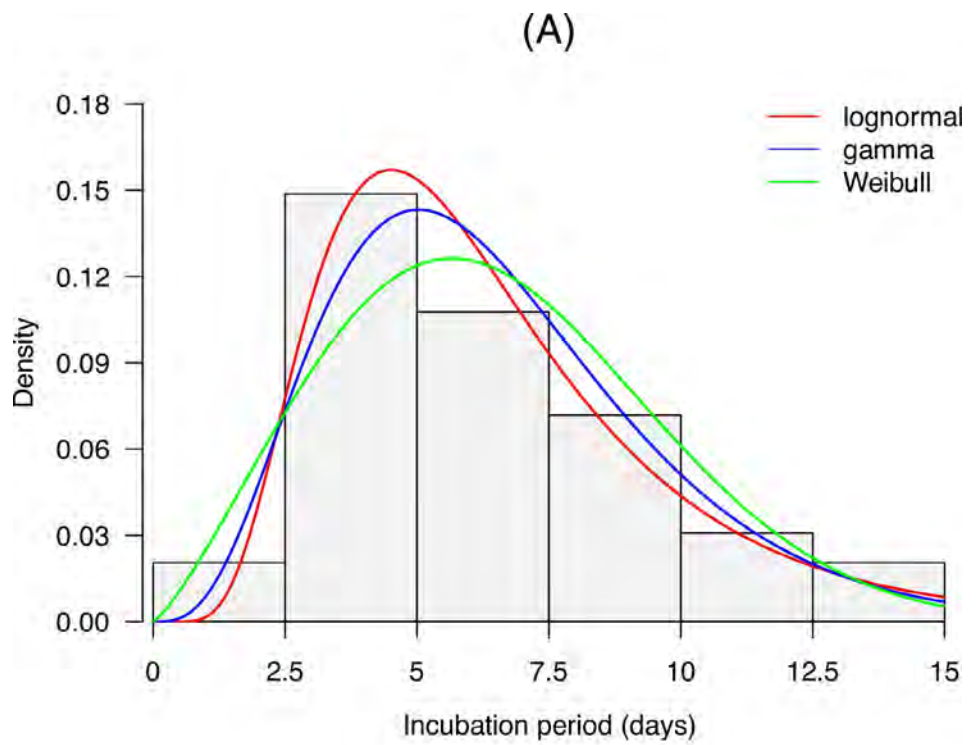


Fig 3. Incubation period and serial interval distributions of the SARS-CoV-2 Delta variant in Guangzhou, China. The blue lines represent the estimated distribution densities. Data of 78 cases and 67 transmission pairs were used to estimate the incubation period and serial interval distributions, respectively.

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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.

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Table 3. Results of an ordinal logistic regression model assessing the association between vaccination status and clinical severity.

Variables	Odds ratio (95% confidence interval)	<i>t</i>	<i>P</i>
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.

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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 pre-existing 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)

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6.9. CoronaVac on teenagers with rheumatic diseases causes three times less adverse effects than vaccines with messenger RNA

A group of researchers from the Medical School of the Istanbul University, in Turkey, concluded that in young people that receive CoronaVac, a vaccine from Butantan and the chinese pharmaceutical Sinovac against Covid-19, the rate of adverse effects after the immunization is three times lower than on those that receive vaccines made with messenger RNA (mRNA). The result was described in a study published at the International Journal of Rheumatic Diseases, and based on a one-year monitoring of 246 teenagers with an average age of 15.

From the 145 participants that received the mRNA vaccine, 107 (74%) experienced adverse events related to the immunization. From the 32 that received CoronaVac, only seven (22%) reported adverse reactions. The most common symptoms were fatigue, headache, myalgia, arthralgia and fever.

Three individuals reported severe adverse events and they required

hospitalization and additional treatment. A 20 year-old girl developed arterial hypertension after the second dose, a 12 year-old girl presented a severe skin rash after the first dose, and a male teenager of 13 years of age developed presyncope due to hypotension after the first dose. None of them had received the CoronaVac vaccine.

Those results prove, once again, that the vaccine from Butantan and Sinovac is the one that has the best safety profile among the immunizers that are currently in use against Covid-19, in adults, elderly, children or teenagers.

In the investigated group there were 126 patients with autoinflammatory diseases, 54 patients with juvenile idiopathic arthritis, 30 patients with connective tissue disease, nine with vasculitis and four with acute rheumatic fever. The control group had 23 healthy teenagers. From the volunteers, 214 patients received the mRNA vaccine, 28 received

CoronaVac and four received both vaccines. Before the immunization, 44 individuals had contracted Covid-19 and recovered, and four of them presented asymptomatic infection and the rest of them had just mild symptoms. The greater part of volunteers used medications regularly before the immunization and kept using it after receiving the vaccine.

According to the researchers, “our study indicates a safety profile acceptable of the vaccines against Covid-19 in our country (Turkey) and encourages the children with rheumatic diseases to be vaccinated”.

During the first days of the pandemic, the children were considered to have asymptomatic or mild cases of Covid-19, in contrast with the adults. However, an increasing number of pediatric cases with multisystem inflammatory syndrome, caused by SARS-CoV-2, have been described with devastating consequences, like hospitalization in intensive care units or even death. Therefore, strategies of vaccination must be well established for children, like it is for adults.

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Early experience of COVID-19 vaccine-related adverse events among adolescents and young adults with rheumatic diseases: A single-center study

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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 inactivated 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 inactivated 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 inactivated SARS-CoV-2 with a 1-month interval. Afterward, the third

to choose their vaccine type, as CoronaVac inactivated 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 inactivated 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%)	-	-
HCCQ, 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 (43.5%)	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, autoimmune inflammatory diseases; ARF, acute rheumatic fever; ASA, acetylsalicylic acid; BD, Behçet disease; bDMARDs, biologic disease-modifying antirheumatic drugs; BS, Blau syndrome; APS, 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; jSRA, 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

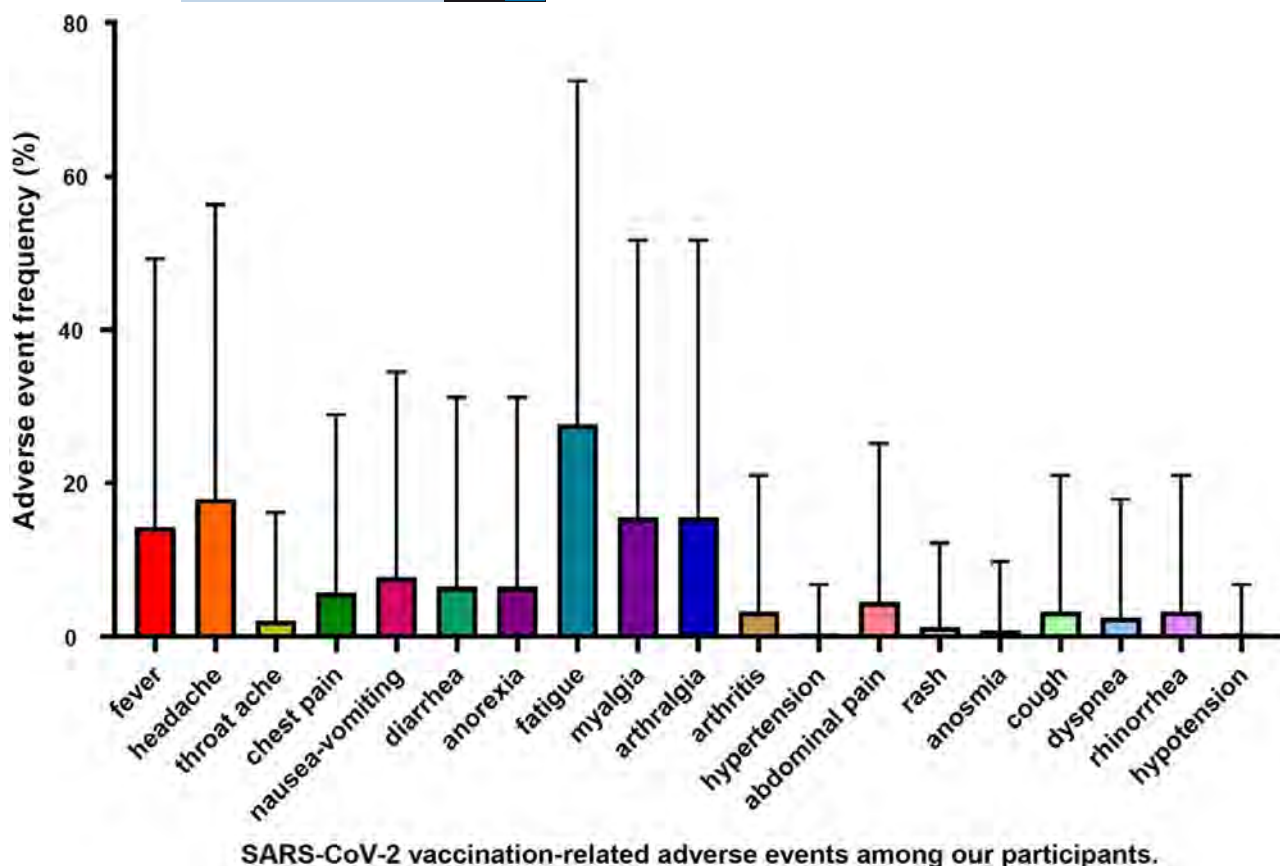


FIGURE 1 SARS-CoV-2 vaccination-related adverse events among our participants

While adverse events were significantly more common among the subjects who received the mRNA vaccine than those who received the inactivated 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

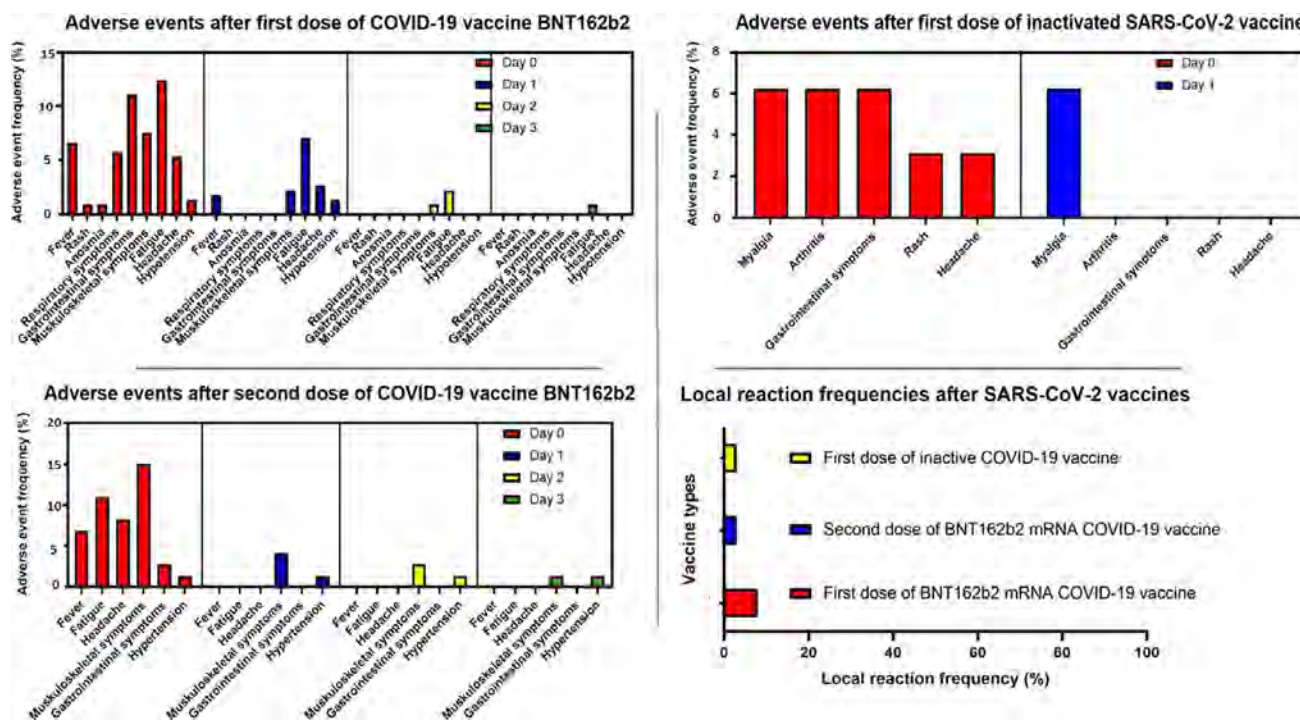


FIGURE 2 Adverse event frequencies according to days and vaccine types

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%)	

^aHealthy 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		P
	Yes (n = 107)	No (n = 139)	
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		P
	Yes (n = 107)	No (n = 139)	
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.

^aTotal of cDMARDs rows are not equal to cDMARDs columns due to several patients being under poly-cDMARDs treatment.

^bFour 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.

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None









CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

All data relevant to the study are included in the article.

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6.10. Study shows that CoronaVac is safe and immunogenic for children aged between seven months and five years

A study of vaccination with CoronaVac made by scientists of the Adolfo Lutz Institute, from the Infectology Institute Emílio Ribas and the State Secretariat of Health from São Paulo concluded that CoronaVac is safe and immunogenic for children. The research was realized with 27 brazilians, with age between seven months and five years, that received the vaccine from Butantan and the chinese pharmaceutic Sinovac in inadvertent way in the cities of Diadema and Itirapina, located in the state of São Paulo. Only one of them presented mild symptoms, without any other important adverse events registered during the monitoring of 30 days.

The children that took part in the study sought for basic units of health

to receive the influenza vaccine but ended up receiving CoronaVac by mistake. The event was immediately communicated to the secretaries of health of each county and, about the adverse event, to the vigilance vaccinal system. The Epidemiologic Vigilance Center of the State Secretariat of Health from São Paulo (CVE) and the Adolfo Lutz Institute attended to the secretaries of Itirapina and Diadema.

The 27 children vaccinated with only one dose were monitored by pediatricians, who collected samples of blood in the first appointment (nine days after the vaccination) and 30 days after the immunization. The only child that reported adverse effects was two years old and presented coryza in the first appointment after the vaccination.

All the children were tested for SARS-CoV-2 S1 serology with total protein Ortho IgG anti-S1 and Cpass, a method that allows a quick detection of total neutralizing antibodies. Five of them had titers of total proteins IgG superior to 1.0 (tests of reagent) between three and nine days after the vaccination. From the total, 19 had the blood collected 30 days after the application and also presented titers of total IgG Spike Proteins superior to 1.0. Four from the five children that presented a positive test in the first appointment were tested again one month after the immunization and presented an increase in the total IgG spike protein anti S1, going from an average of 10,4 to 20,5.

The objectives of the study were to describe the response of the health public system to a programmatic error and to monitor the safety, tolerability and seroconversion of the vaccine through the detection of the total amount of IgG antibodies against the Spike protein after the vaccination of children with CoronaVac.

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Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in inadvertently vaccinated healthy children

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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 Butantan¹. Over 40 million doses of CoronaVac had already been administered by the end of June 2021 all over the country².

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The vaccination error was promptly reported to the health department of each municipality and, in relation to adverse events, to the vaccination surveillance system. The Epidemiological Surveillance Center of Sao Paulo State (CIEVE) and the Adolfo Lutz Institute assisted the health departments of Itirapina and Diadema. The objectives were to describe the public health response to a programmatic error and to monitor the vaccine safety, tolerability and seroconversion by detecting the total amount of IgG antibodies against SARS-CoV-2 S1 spike protein after the vaccination of children with CoronaVac.

MATERIALS AND METHODS

The children who had been inadvertently vaccinated with CoronaVac (Sinovac Life Sciences, Beijing, China) were monitored by pediatricians in primary health care units for 30 days, to receive medical assistance if any sign or symptom appeared. Reports of their health conditions were sent to the health department of each municipality. Three visits were scheduled for medical evaluation, right after the event recognition (error in the vaccine used), at 15th and 30th day after vaccination. To inform the families and local health workers caring for these children of their serological status, two registered assays, available at State public laboratories were used. Blood samples were taken on the first medical evaluation (3-9 days after the event) and on the 30th day after the vaccination event. The presence of antibodies for SARS-CoV-2 were detected using (i) a chemiluminescent microparticle assay (VITROS[®] Anti-SARS-CoV2, Ortho Clinical Diagnostics, United Kingdom) which detects the domain of the S1 (spike) antigen, considering cross-reactive for SARS-CoV-2 antibodies samples with titers >1.0 and; (ii) the evaluation of antibodies able to interfere with the BD-ACE2 interaction (RBI), measured by cPass (SARS-CoV-2 Neutralization Antibody Detection kit, GenScript, SA), both test performed following the manufacturer's instructions. The test was considered positive for the presence of neutralizing antibodies for SARS-CoV-2 when a inhibition titer $\geq 20\%$ is obtained, and samples are assigned as presenting with low inhibition when percentages from 5% to 20% inhibition are detected.

All clinical information and laboratory tests results were registered in each case, reporting the clinical manifestations of adverse events to the health departments and to the programmatic error surveillance system.

The approach to these children occurred only after the detection 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 years³.

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 older⁴. A Phase 1-2 study evaluated children and adolescents

Table 1 - Demographic and serological results from children inadvertently vaccinated with CoronaVAc (one dose), Sao Paulo State, Brazil, 2021.

Sex	Age (months)	DV 1	DV 2	S1 Ab 1	S1 Ab 2	RBI 1	RBI 2
Female	22	4	NA	0.01	NA	5.00	NA
Female	28	4	30	0.00	6.49	19.61	30.95
Female	42	4	30	3.11	19.00	39.90	NA
Female	69	4	NA	0.01	NA	NA	NA
Female	44	4	30	0.00	7.53	-6.89	45.22
Female	30	4	NA	11.30	NA	NA	NA
Female	3	6	30	0.01	7.73	9.07	62.34
Female	60	7	NA	0.01	NA	NA	NA
Female	7	3	33	0.00	10.10	21.83	64.87
Female	37	3	33	0.00	3.03	3.60	33.04
Female	60	3	33	0.00	7.94	8.73	51.00
Female	54	9	NA	0.02	NA	NA	NA
Female	52	4	NA	0.01	NA	-0.69	NA
Female	31	4	NA	0.00	NA	NA	NA
Female	23	4	30	0.00	3.77	NA	22.05
Female	22	4	NA	0.03	NA	NA	NA
Female	60	4	30	5.17	20.50	91.50	96.8
Female	31	4	30	0.00	3.00	27.12	35.84
Female	46	4	30	0.00	10.20	-10.54	38.68
Female	10	4	30	0.00	8.90	22.99	68.12
Female	13	4	30	0.00	11.20	22.50	68.96
Female	49	4	30	0.01	4.19	13.21	35.79
Female	35	4	30	0.03	5.48	23.48	38.06
Female	32	4	41	0.01	9.73	NA	NA
Female	18	3	33	19.00	24.10	97.07	NA
Female	54	5	34	0.17	6.95	19.48	57.98
Female	23	9	30	13.30	18.60	97.36	NA

DV 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; S1 Ab 1= antibody titers against the SPIKE domain S1 at the time of the 1st blood sampling ; S1 Ab 2 = antibody titers against the SPIKE domain S1 at the time of the 2nd blood sampling ; RBI 1 = percentage of receptor binding inhibition at the time of the 1st blood sampling ; RBI 2 = percentage of receptor binding inhibition at the time of the 2st blood sampling ; NA = not available.

events were non-severe, and the most common reactions were pain at the injection site and fever³.

All tested children showed an increase in total S1 spike protein IgG antibodies 30 days following the vaccination. Although some children already had antibodies at the time of the initial blood collection, presumably due to previous symptomatic, unrecognized infection by SARS-CoV-2. When these previously positive children were tested 30 days after the vaccination, they showed an increment in IgG binding antibody units at the second blood sampling. As no infection 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 antibodies⁵. 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

limited to a serological response to S1 antigens, either total IgG to the viral S1 protein binding inhibition to the major SARS-CoV-2 receptor, the data suggest an anti-spike response after one dose of the vaccine. In other words, one dose of CoronaVac was immunogenic in children³.

Wrong vaccine administration is the most reported vaccination error^{7,8}. CoronaVac and influenza vaccines used in the Brazilian public health system come from the same local producer (Butantan) and they have the multiple dose presentation, which could favor the confusion. However, the label and the color of the bottle cap are different. The current high number of different vaccines available in the Brazilian immunization schedule demands well trained health professionals. Vaccination errors may harm patients and cause a negative impact on the population's confidence in vaccination, which in turn will negatively impact the vaccination coverage⁸.

This study has some limitations. Firstly, it is a response to an unexpected event, justifying the small sample size that does not allow us to rule out the occurrence of rare adverse events or even to definitely conclude on the duration of the seroconversion observed after the first dose. Secondly, children did not receive the second dose and were not evaluated after the end of the proposed immunization. Thirdly, the cellular immunity was not evaluated. Finally, the monitoring period (30 days) was short to determine long-term immunogenicity and also for a complete evaluation of safety.

Children infected with SARS-CoV-2 mainly have mild disease or are asymptomatic, when compared with adults. However, a small number of children, especially those with health comorbidities, might be at risk of severe COVID-19^{9,10}. Furthermore, the SARS-CoV-2 infection can lead to a serious, although rare complication called the multisystem inflammatory syndrome in children¹¹. Finally, children can be transmitters of SARS-CoV-2 in communities¹². A vaccine against SARS-CoV-2 for children and adolescents will contribute decisively to the control of the COVID-19 pandemic. Our investigation suggests that CoronaVac is well tolerated and safe and can induce humoral responses in children, but proper safety and effectiveness studies must be performed before expanding the vaccination to young children.

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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.

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6.11. Systematic revision of scientific studies proves the safety and efficacy of CoronaVac for children and teenagers

Chinese researchers made a systematic revision about controlled and randomic studies, case studies and case series studies with the objective of estimating the safety, immunogenicity and efficacy of the vaccination on children and teenagers against Covid-19. The research was published in the journal *Vaccines* and conducted by scientists of the Medical University of Chongqing, the Lanzhou University and the National Center of Medical Research on Health and Infant Diseases of China.

The researchers investigated studies published until July 23, 2021, in the platforms PubMed, Web of Science, in the database regarding Covid-19 of the World Health Organization (WHO) and in the China National Knowledge Infrastructure (CNKI).

The revision included eight published studies, involving a total of 2.852 children, and 28 clinical studies that are still ongoing. One of the main researches analyzed was the randomic clinical trial of phase 1 and 2 of the use of CoronaVac among children from three to 17 years of age, that was made in China. The rest of the papers regard mRNA vaccines.

According to the revision, the clinical trial of CoronaVac demonstrated that the vaccine has a good safety profile and is immunogenic for children and teenagers. Regarding safety, the majority of the adverse events were mild or moderate, such as pain in the area of the injection, fatigue, headache and chest pain. About the immunogenicity, on both phases 1 and 2, the seroconversion of neutralizing antibodies after the second dose was 100%.

“Our study found high levels of immunogenicity and vaccinal efficacy in children and teenagers. It is a clear indication that the vaccines are effective, and the random controlled studies did not report problems with safety”, concluded the researchers.

The vaccine is the most efficient way to prevent and control infections by Covid-19, besides stimulating the immunological system to produce antibodies. Promoting vaccination to children and teenagers is crucial to stop the propagation of coronavirus, since that group represents a quarter of the mundial population.

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Systematic Review

Safety, Immunogenicity, and Efficacy of COVID-19 Vaccines in Children and Adolescents: A Systematic Review

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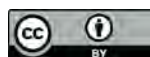


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Abstract: **Aim:** To identify the safety, immunogenicity, and protective efficacy of COVID-19 vaccines in children and adolescents. **Methods:** We conducted a systematic review of published studies and ongoing clinical studies related to the safety, 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.

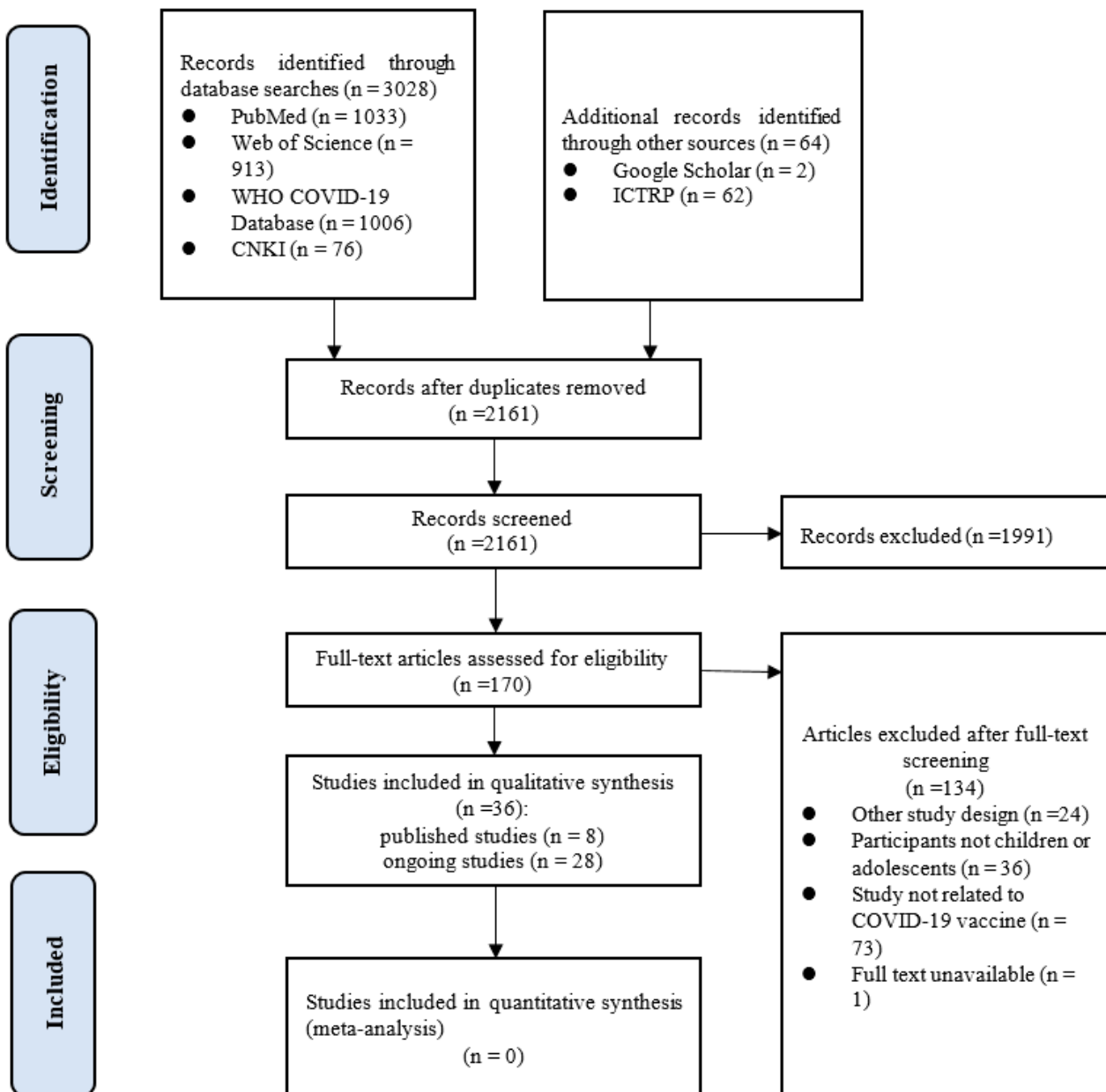


Figure 1. Study selection process (WHO: World Health Organization; COVID-19: coronavirus disease 2019; CNKI: China National Knowledge Infrastructure; ICTRP: International Clinical Trials Registry Platform).

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								Study
Selection bias		Performance bias	Detection bias	Attrition bias	Reporting bias		Other bias	
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]
Methodological quality in the case series and case reports assessed by Murad et al. checklist								Study
Selection	Ascertainment		Causality			Reporting		
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 Phase 1/2 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.

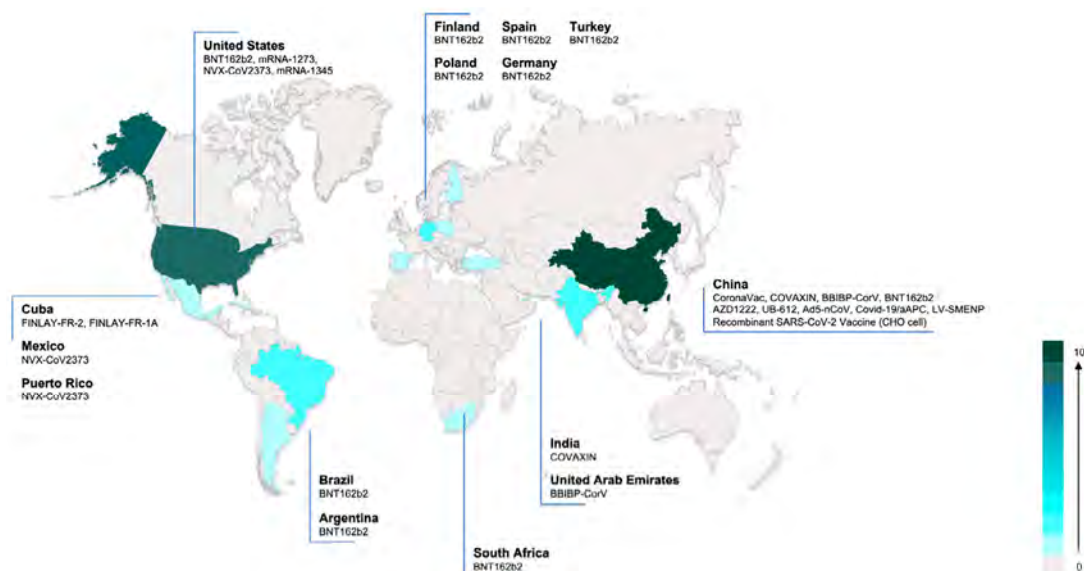


Figure 2. Ongoing interventional COVID-19 vaccine trials in children and adolescents worldwide. Color in the figure indicates the number of ongoing vaccine trials in each country.

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 ≥ 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 indicator 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.

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6.12. Study of more than ten million Chileans over the age of 16 shows that CoronaVac effectiveness is over 86%

The effectiveness of CoronaVac among adolescents has been proven since September 2021, when Chilean researchers published the article “Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile” in The New England Journal of Medicine, one of the most prestigious journals in the world. The study, conducted between February and May 2021, with 10.2 million people, investigated the effectiveness of the vaccine in the “real world” against Covid-19 cases and in combating the SARS-CoV-2 variants circulating in the country - gamma and alpha, mainly.

The cohort study (observational research that follows individuals over a period of time to determine characteristics and evolution of the group) included participants over the age of 16 who are registered with the National Fund of Health (FONASA), Chile’s national health program that covers about 80% of the population. The vaccination schedule applied in the country is two doses of CoronaVac with a 28-day interval.

The research showed that the protection of the Butantan and

Sinovac vaccine was 65.9% against Covid-19 infections, 87.5% against hospitalizations, 90.3% against Intensive Care Unit (ICU) admissions, and 86.3% against deaths.

A total of 708,676 young people aged 16 to 19 years, equivalent to 7% of the total cohort volunteers, participated in the study. Of these, 8,192 (1.2%) received one dose of CoronaVac and 30,033 (4.2%) received two doses. The remaining 670,451 consisted of a control group or people who had had Covid-19 (14,871). It is worth noting that in Chile, as in Brazil, vaccination was initiated by the elderly, who are considered more vulnerable to Covid-19.

The Andean country has the highest rates of testing for Covid-19 in Latin America and a standardized public information system for statistics vital to the study. At the time, the Chilean Ministry of Health had already used 13.98 million doses of CoronaVac since the vaccination campaign began in February.

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Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile

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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., E.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 CIRAR Azrieli Global Scholars Program, CIRAR, 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 32461, Las Condes 7580943, Chile, or at rafaelaraos@udd.cl.

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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 Israel¹⁵ and Scotland¹⁶ 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 coun-

tries 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 vaccine²² were carried out in China among participants 18 to 59 years of age²³ 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-

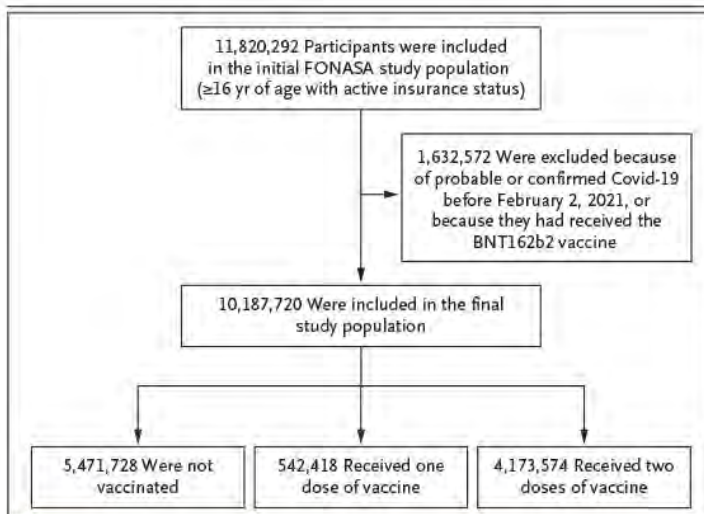


Figure 1. Study Participants and Cohort Eligibility.

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

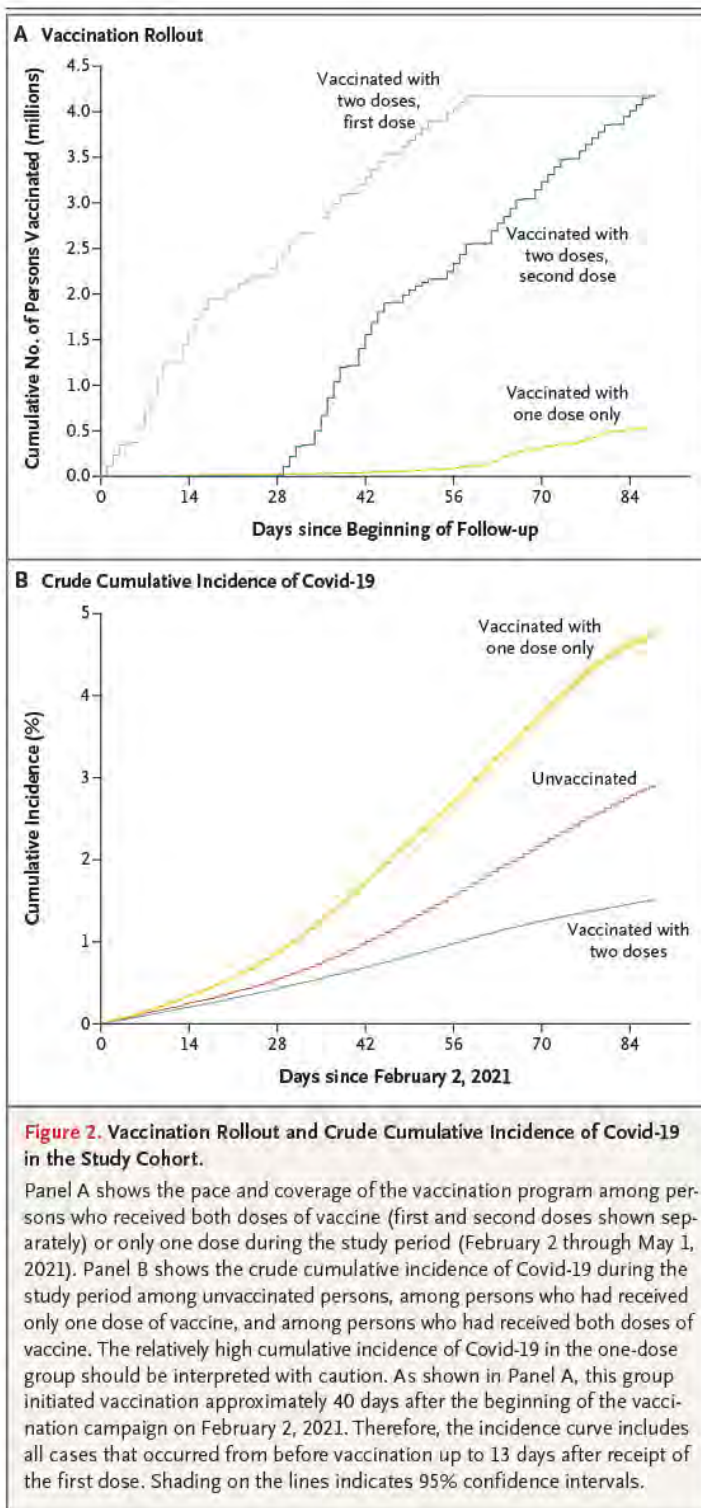
There were approximately 615 million person-days in the unvaccinated group, 70 million person-days in the partially immunized group, and 92 million

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	
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	15.1	

* 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.



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 No. of Person-Days	Persons with Covid-19		Vaccine Effectiveness (95% CI)		
		No. of Persons	Incidence Rate <i>no. of events/ 1000 person-days</i>	Analysis Adjusted for Sex and Age	Analysis Adjusted for All Covariates†	Stratified Analysis‡
				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 No. of Person-Days	Persons with Covid-19		Vaccine Effectiveness (95% CI)		
		No. of Persons	Incidence Rate <i>no. of events/ 1000 person-days</i>	Analysis Adjusted for Sex and Age	Analysis Adjusted for All Covariates*	Stratified Analysis†
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 effec-

tiveness 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 disease³ 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 con-

sidered 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 Brazil⁴²), 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.

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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>.

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6.13. Child mortality from Covid-19 is much higher in poor countries, where vaccination of the very young is not planned

The mortality of children from Covid-19 is much higher in poor countries than in rich countries, i.e., precisely in those nations that have not yet included this public in their vaccination programs. The inequality in vaccine distribution and medical care explains the problem and opens the discussion of when and how to include this population in the Covid-19 vaccination, wrote researchers Beate Kampmann and Uduak Okomo, from the London School of Hygiene & Tropical Medicine, in an article in the scientific journal *The Lancet*.

The researchers raise the thesis based on the results of a meta-analysis (a statistical method that analyzes data from two or more studies) that concluded that 91,5% of global child and adolescent deaths from Covid-19 were reported in low and middle income

countries, while 83.5 percent of the infected pediatric population was from these countries.

The robust study, which reviewed more than 16,000 scientific papers and 225 national reports from 216 countries, pointed out that the death rate was significantly higher in low and middle income countries than in rich countries: 2.77 versus 1.32 per million children. The data compiled by researchers at the University of Toronto was published in the scientific journal *PLOS One*.

“This great inequality prevents low and middle income countries from not only avoiding deaths and serious illness, but also deploying vaccines as tools to interrupt SARS-CoV-2 transmission. Including children and adolescents will not be a priority in these poorer countries for a long time because

of the severe shortcomings in vaccine distribution,” they describe in the article. Given the data, the researchers point out that the protection of children against Covid-19 will depend more on national factors and public policies, which may or may not include access to vaccines for this group.

“The impacts of Covid-19 vaccination in children and adolescents on transmission dynamics will vary nationally, taking into account epidemiological circumstances, the emergence of new SARS-CoV-2 variants, and contact mitigation strategies in different places and roles,” they add.

Such inequality blurs the results of studies with inactivated virus vaccines, such as CoronaVac, and messenger RNA vaccines, which have been

shown to be safe and immunogenic for children and adolescents, in the researchers’ opinion.

“There is no reason to believe that vaccines should not be equally protective against Covid-19 in children and adolescents as in adults. More than 30 international trials recruit children and adolescents from six months old to assess safety, immunogenicity, dosing, and distribution,” they explain.

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COVID-19 vaccines for children in LMICs: another equity issue



Given the success of COVID-19 vaccines in preventing death and severe disease in adults¹ 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 vaccine⁵ 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

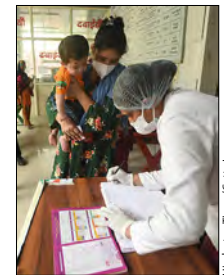
Countries are also still calculating what indirect benefits for reduced SARS-CoV-2 transmission in schools and the wider community could be achieved by vaccinating CYP. With children now recognised as part of the chains of community transmission,⁴ the discussion about a CYP vaccine programme was perhaps 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,



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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.

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6.14. CoronaVac is safe and generates a strong immune response in children and teenagers, confirms study

CoronaVac, a vaccine against Covid-19 developed by the Chinese biopharmaceutical Sinovac Biotech and produced in Brazil by Butantan, is safe for the population from three to 17 years of age and may induce a strong production of antibodies in the pediatric group. The conclusions were obtained in the clinical trials of phase 1 and 2 conducted by Sinovac with the application of CoronaVac on children and teenagers. The results were published in the scientific periodic journal *The Lancet Infectious Diseases*.

This is the first study in the world to evaluate the use of a vaccine against Covid-19 in a population from three years of age. “Children and teenagers with Covid-19 usually have mild infections or are asymptomatic in comparison to adults. Besides that, a small number can still be at risk of a severe disease and this population can still transmit the virus to other people. Therefore, it’s vital to test the safety and efficacy of the vaccine against Covid-19 in the younger groups”, said the general manager of Sinovac, Gao Qiang, in a communication published in the website of the pharmaceutical.

The randomized, controlled and double blinded study evaluated 550 children (71 in phase 1 and 479 in phase 2) between three and 17 years old to measure the safety, the tolerability and the immunogenicity

of the application of two doses of CoronaVac with a gap of 28 days.

A group received the vaccine while the other received placebo with aluminum hydroxide, a non-harmful adjuvant that is present in the formula of the immunizer. The analyses revealed that the vaccine was capable of generating antibodies in 96% of the volunteers 28 days after the second dose. In phase 1, none of the participants had neutralizing antibodies against the SARS-CoV-2 and, 28 days after the vaccination, 100% of them presented antibodies.

In phase 2, some of the volunteers received two applications with a minor dosage (1,5 µg) and others received a higher dosage (3 µg). While in the first group 95% of the participants presented antibodies, this number was 100% in the second group. That is the reason why the researchers decided to keep using only the higher dosage during the clinical trial of phase 3, which is still ongoing.

The adverse events were from mild to moderate and the most common reactions reported were local pain and fever, that disappeared after 24 hours. 27% of the participants reported collateral effects. There was only one severe adverse event, not associated with the vaccine - a child had pneumonia after receiving placebo.

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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

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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 Zhanhuang (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.

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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

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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 Zhanhuang (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>

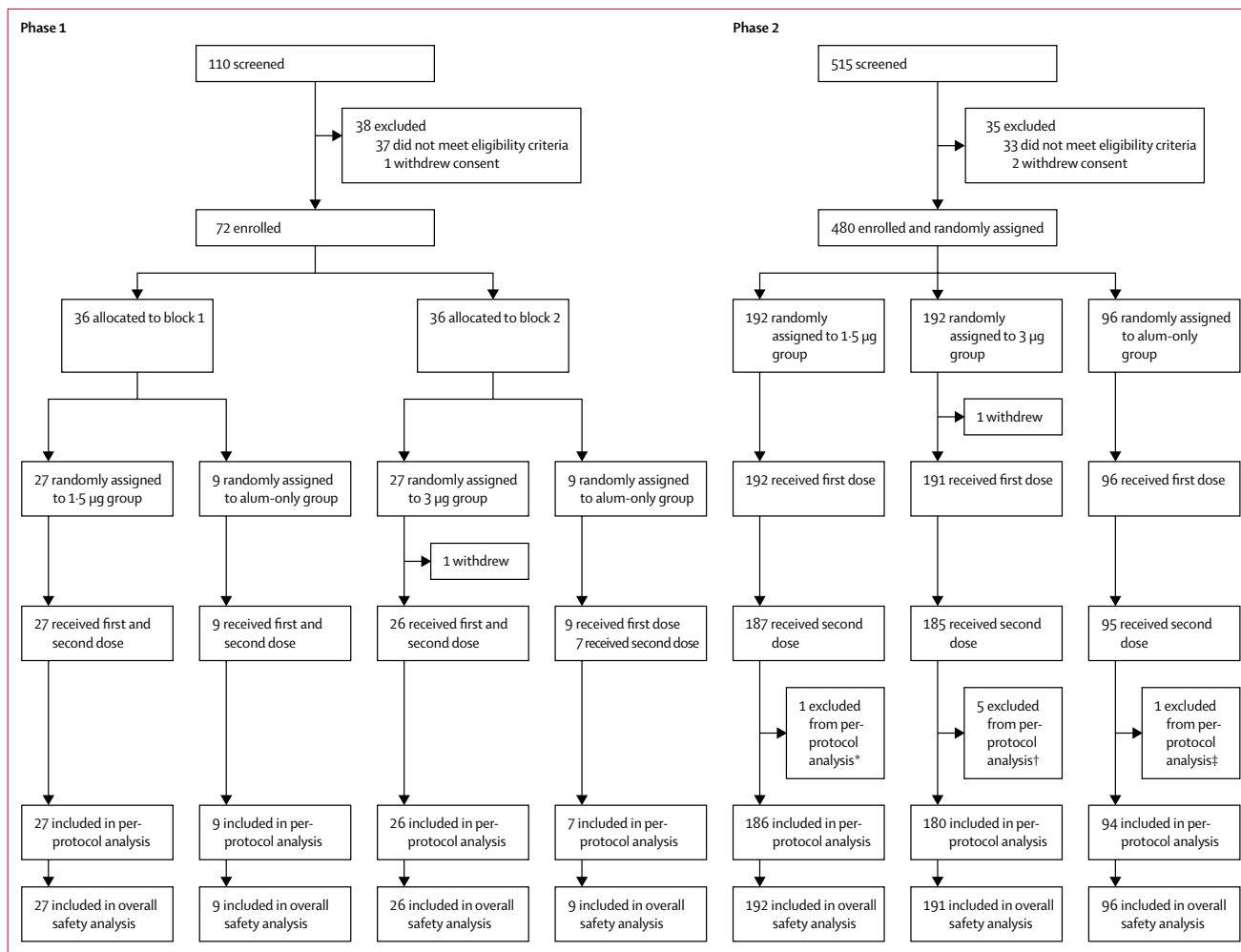


Figure 1: Trial profile

*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 × 10⁵ 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

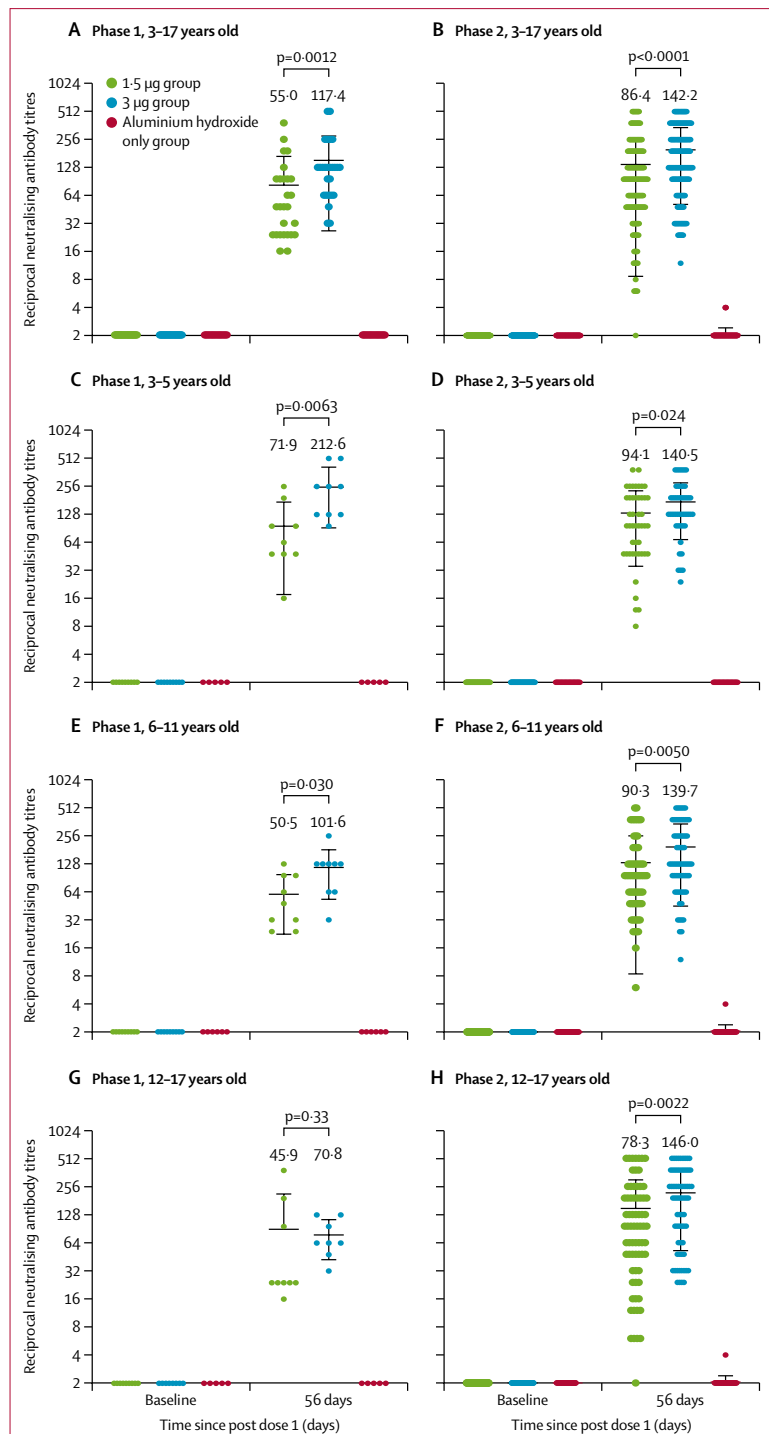


Figure 2: Antibody titres of neutralising antibodies to live SARS-CoV-2 induced after two doses of CoronaVac or aluminium hydroxide diluent only in phase 1 and phase 2 trials

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

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7. Booster dose multiplies antibodies

7.1. CoronaVac booster protects by 98% against severe cases and death from Covid-19, says Hong Kong study

A study carried out in Hong Kong during the SARS-CoV-2 omicron outbreak in the first quarter of 2022 showed that the administration of three doses of CoronaVac provided 98% protection against severe cases and death from Covid-19, mainly among the elderly. The paper was peer-reviewed and published in *The Lancet Infectious Diseases* and conducted by researchers at the University of Hong Kong School of Public Health and School of Medicine. The results were previously published in preprint format.

Until December 2021, Hong Kong excelled in controlling the pandemic due to the restrictive social isolation imposed by the government. But at the beginning of January 2022, the outbreak of omicron sublineage BA.2 exploded in the city, resulting in 741,000 cases of Covid-19 and 8,875 severe cases or deaths.

As of mid-April 2022, 61% of the population aged between 40 and 59 had had three doses of the vaccine. This percentage was 39% in

the range between 70 and 79 years and 15% among the elderly aged 80 years and over.

The administration of two doses of CoronaVac showed an efficacy of 91.7% in preventing severe cases of Covid-19 in adults aged 20 to 59 years. Among the elderly aged 60 to 69 years, the efficacy of two doses reached 79.3%, and 74.3% among those aged 70 to 79 years.

In the case of the booster dose, the results were even better. “We estimate that three doses offered very high protection against serious illness (97.9%) and death (98.6%) in all age groups,” the study authors state.

Specifically among the elderly, three doses of CoronaVac showed an efficiency in preventing severe cases and death of 97.4%, 95.4% and 97.3%, in the groups aged 60 to 69 years, 70 to 79 years, and 80 years or older, respectively.

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Vaccine effectiveness of one, two, and three doses of BNT162b2 and CoronaVac against COVID-19 in Hong Kong: a population-based observational study



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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-naïve 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.

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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

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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-naïve 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-naïve 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.

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 $(1 - \text{IRR}) \times 100\%$. We performed sensitivity analyses calculating incidence per calendar

For the Department of Health Dashboard see <https://www.covidvaccine.gov.hk/en/dashboard>

See Online for appendix

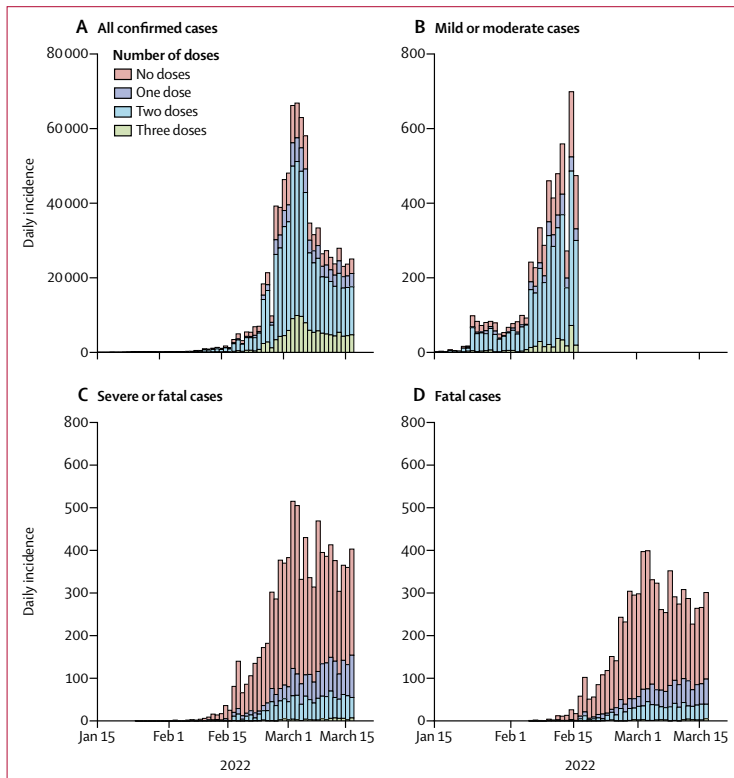


Figure 1: Daily incidence of cases and deaths by vaccination status
 (A) All confirmed COVID-19 cases. (B) Mild or moderate cases in the early part of the fifth wave before Feb 15, 2022. (C) Severe or fatal cases. (D) Deaths throughout the fifth wave in Hong Kong. Severe disease was defined as having 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

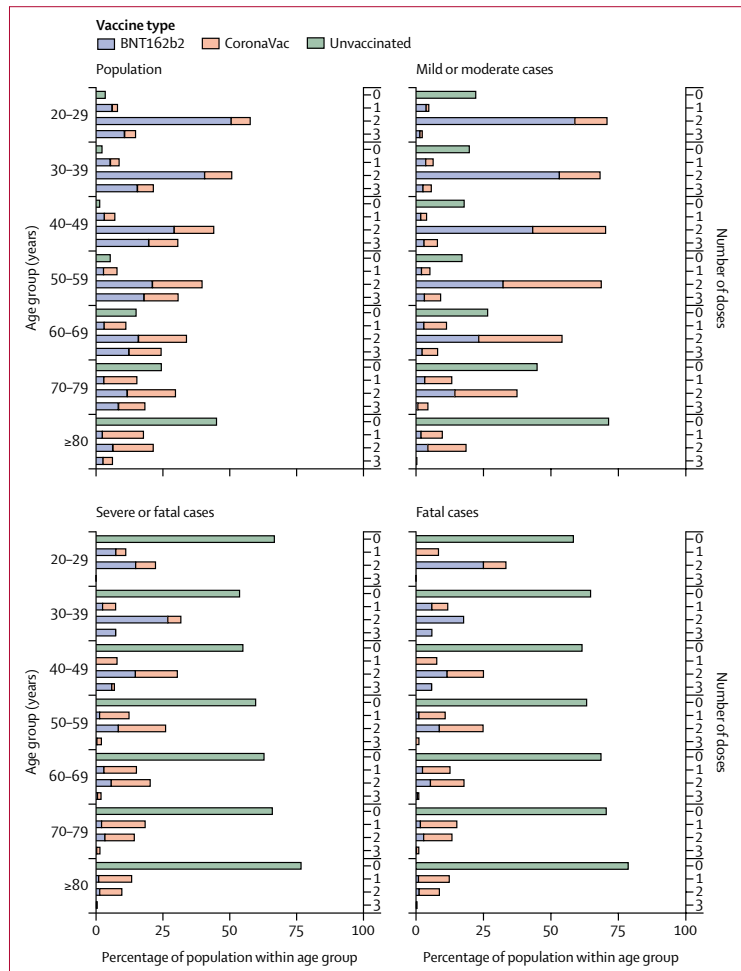


Figure 2: Vaccine status, age group, and vaccine type

(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-naïve 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-naïve, 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 study²⁵ 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 USA²⁶ 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 Malaysia³⁵ 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.

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7.2. Chinese study indicates that CoronaVac booster dose multiplies neutralizing antibodies by 78 times

Research carried out in Beijing, China, showed that the CoronaVac booster dose increased the level of neutralizing antibodies by almost 80 times, specifically raising the IgG antibodies of the IgG1 and IgG3 subclasses – something that had not yet been observed. The study was published in the journal *Immunology* and conducted by researchers from the Beijing Centers for Disease Control and Prevention and the Capital Medical University.

The study included 174 adults ages 18 to 59 who received two doses of CoronaVac. Of these, 158 received the third dose after one year of primary immunization and were followed up for 15 months. After 10 days of the booster, seroconversion reached 100% and remained so in the following months. Neutralizing antibodies increased 78-fold, reaching a peak of 290.6 after three weeks.

The researchers also evaluated, for the first time, antibody subclasses, which may serve as important determinants of vaccine efficacy.


Neutralizing antibodies are mainly composed of IgG, IgA and IgM, among which IgG is the most abundant and multifunctional. IgG has 4 distinct subclasses: IgG1, IgG2, IgG3 and IgG4.

After the booster dose, levels for IgG1 multiplied 21-fold, increasing from 21.7 to 458.4. The levels for IgG3 had an 8-fold increase from 29.5 to 241.8. The proportion of IgG1 and IgG3 was correlated with the high level of antibodies capable of neutralizing the virus.

According to the researchers, it has already been shown that the humoral immune response of individuals recovered from Covid-19 consists mainly of IgG1-producing memory B cells. “It remains to be investigated whether immunization with inactivated vaccines [such as CoronaVac] is directly related to the presence of memory B cells.”

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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

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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 administered 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

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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 $> 37.0^{\circ}\text{C}$ 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/

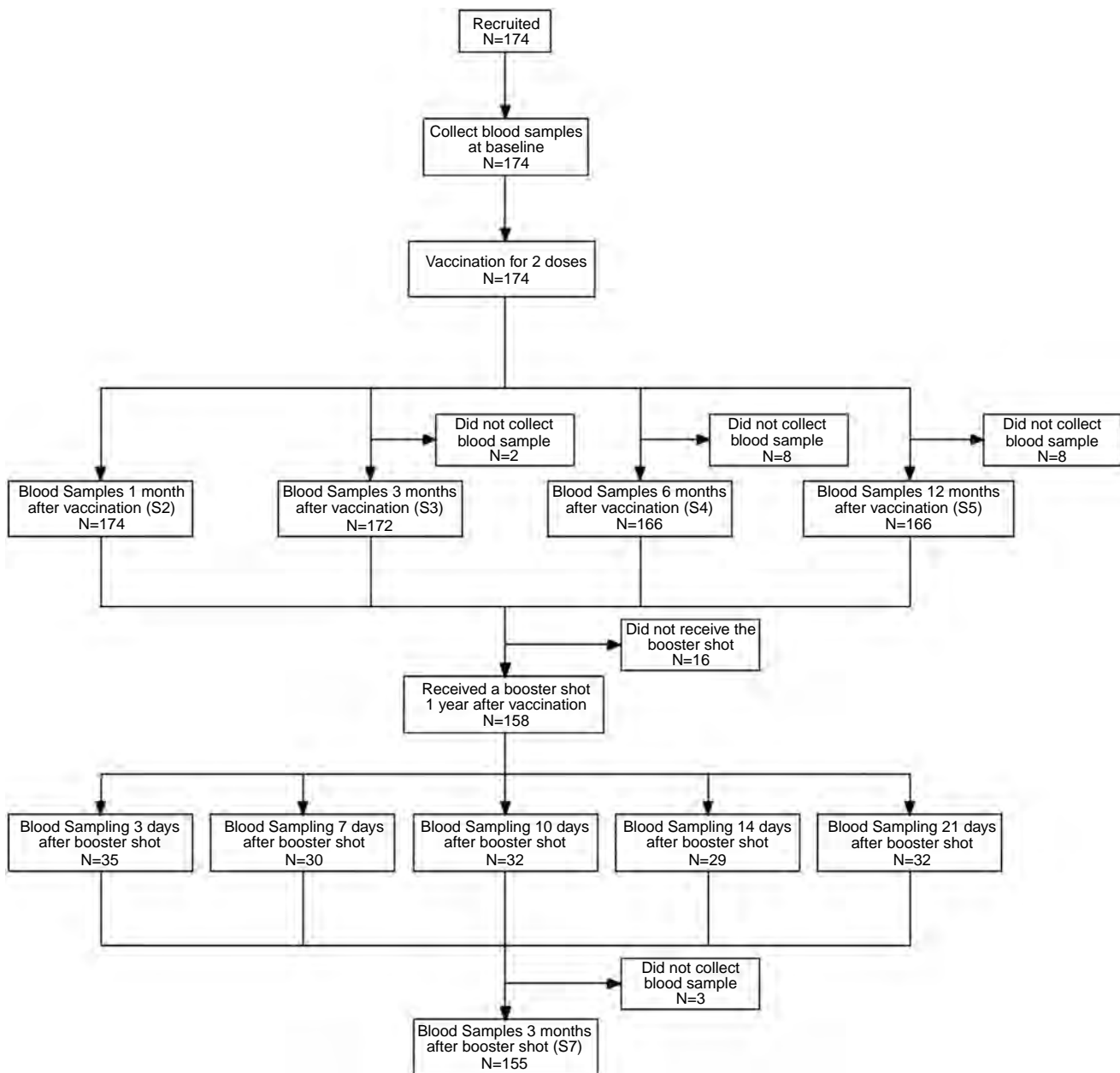


FIGURE 1 Study design and the sample collection

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 × 10⁵ 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 $S/CO \times$ 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 \times dilution multiple was the antibody level corresponding to the sample.

Statistical analysis

IgG subclass levels were presented as $S/CO \times$ 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

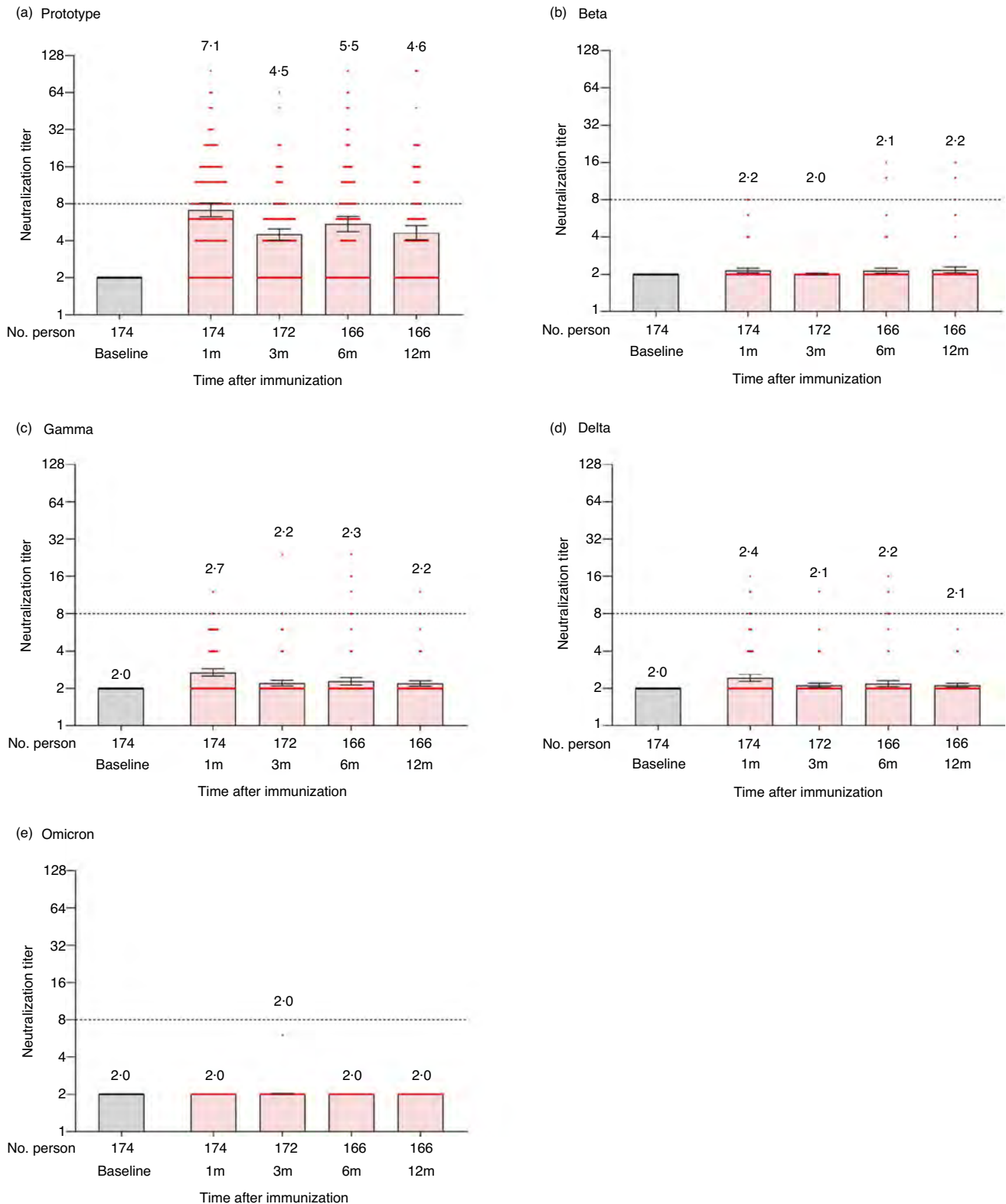


FIGURE 2 Neutralizing antibodies against the wild-type strain and variants of SARS-CoV-2 after 2-dose primary immunization. The results of neutralization assays against SARS-CoV-2 wild-type strain (a), Beta variant (b), Gamma variant (c), Delta variant (d), and Omicron variant (e). Each dot represents 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)

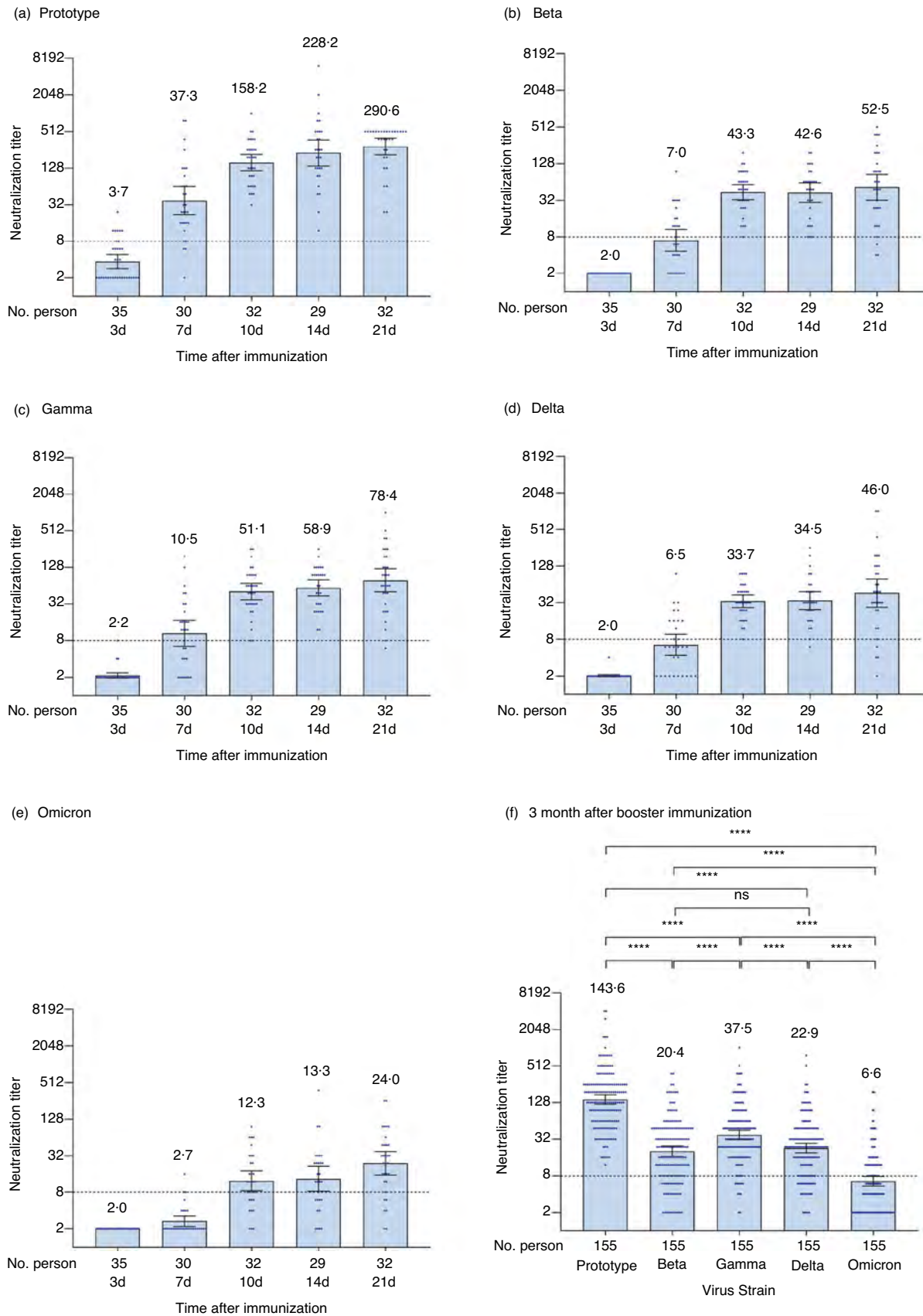


FIGURE 3 Legend on next page.

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).

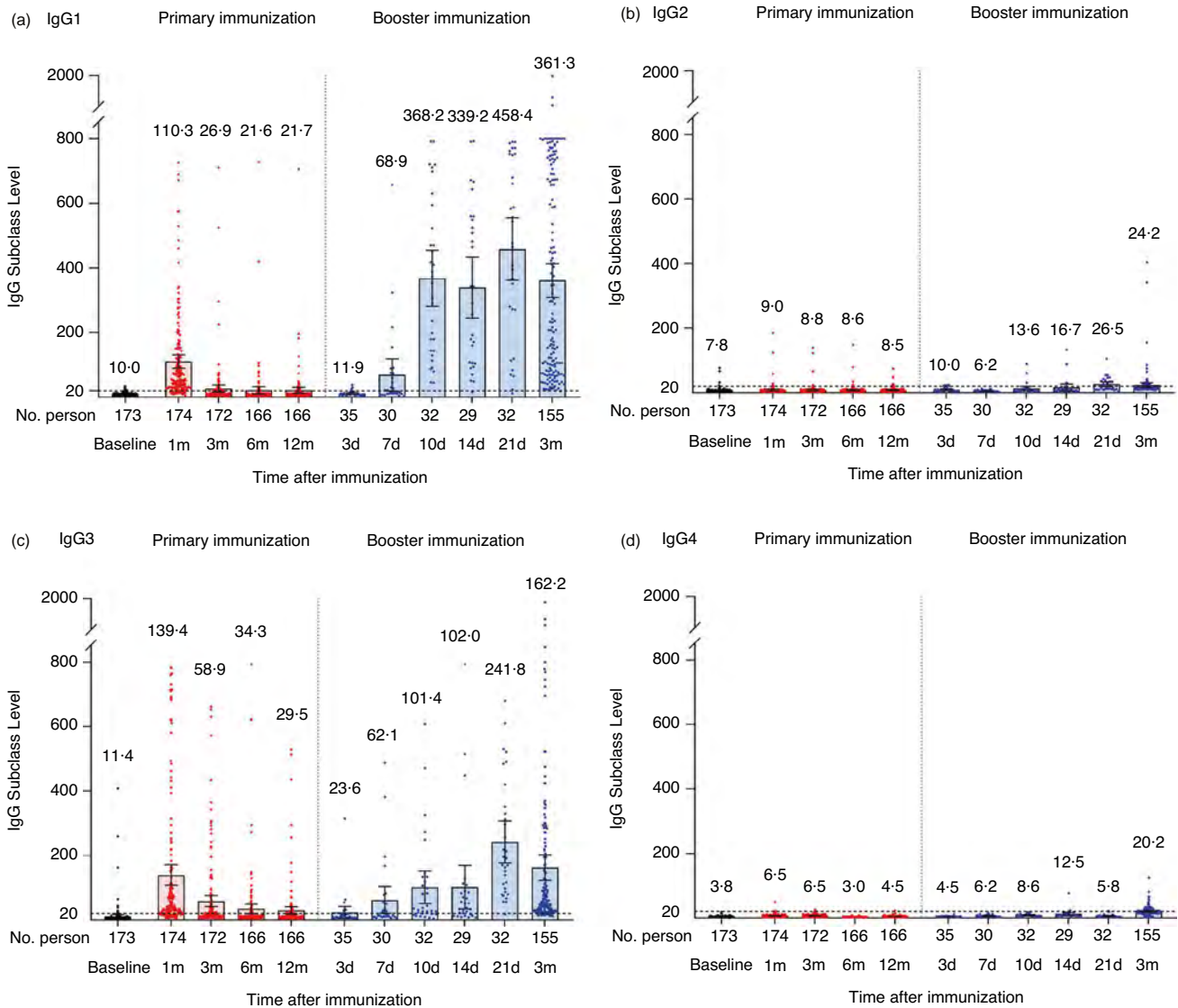


FIGURE 4 IgG subclasses at the different time points after 2-dose primary immunization and the booster shot. The levels of IgG1 (a), IgG2 (b), IgG3 (c) and IgG4 (d) after 2-dose primary immunization and the booster shot. Each dot represents the IgG subclass level of an individual. The numbers above the 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

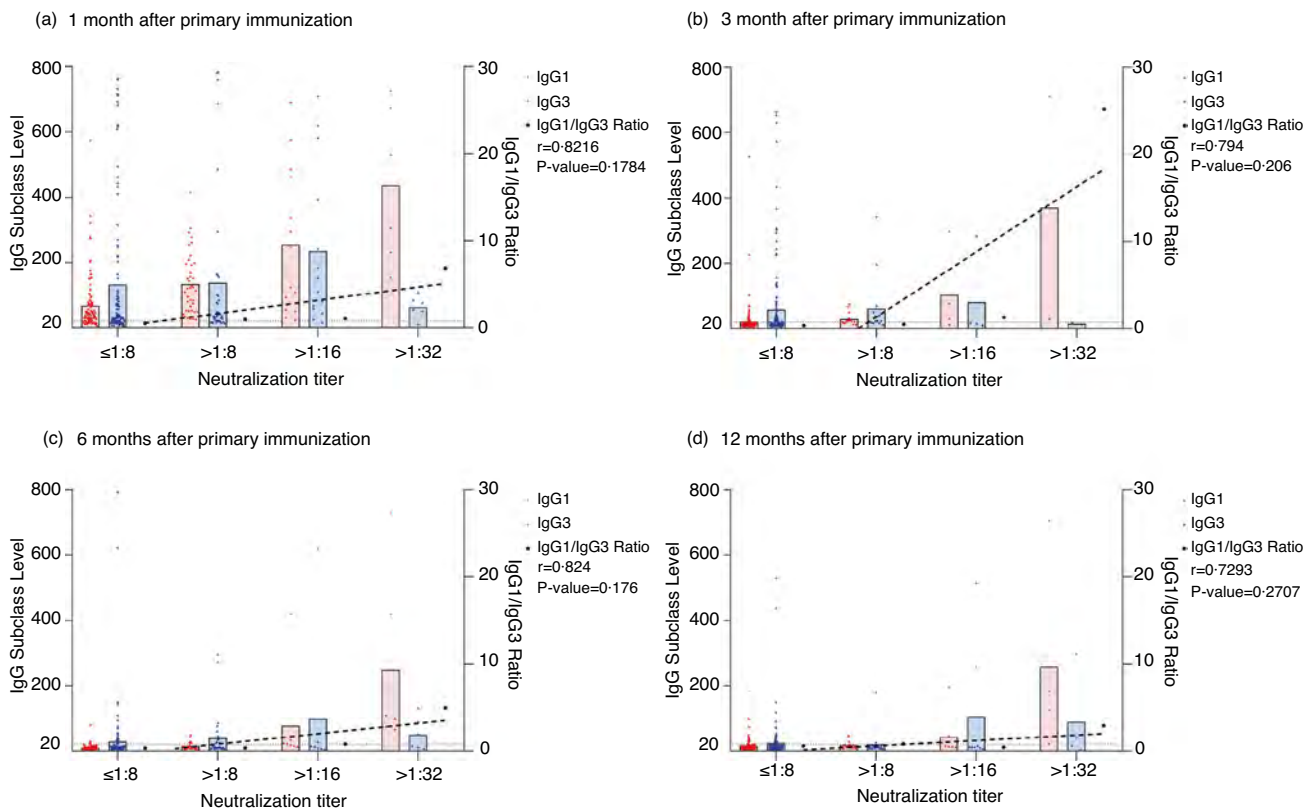


FIGURE 5 IgG1 and IgG3 distribution after 2-dose primary immunization. IgG1, IgG3 and IgG1/IgG3 ratio was grouped by titres of the neutralizing antibodies at 1 month (a), 3 months (b), 6 months (c) and 12 months (d) after 2-dose primary immunization. The dotted line represents the linear trend between 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

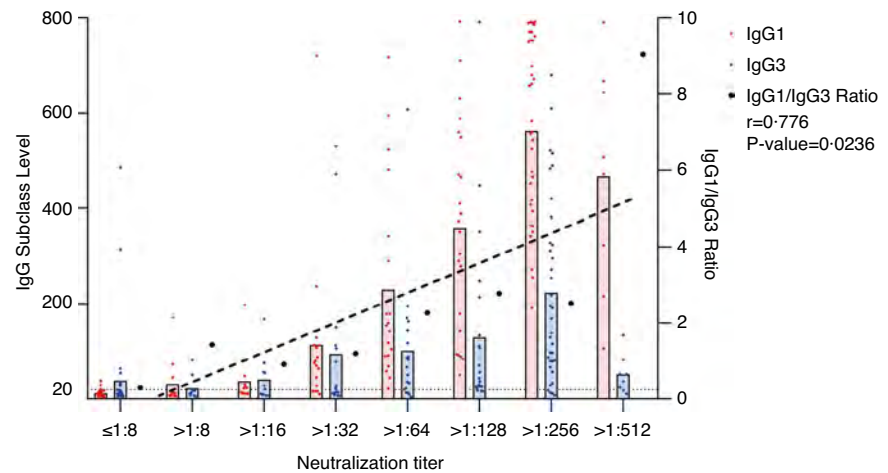
SARS-CoV-2 RBD-specific IgG subclasses after 2-dose primary immunization and booster shot

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

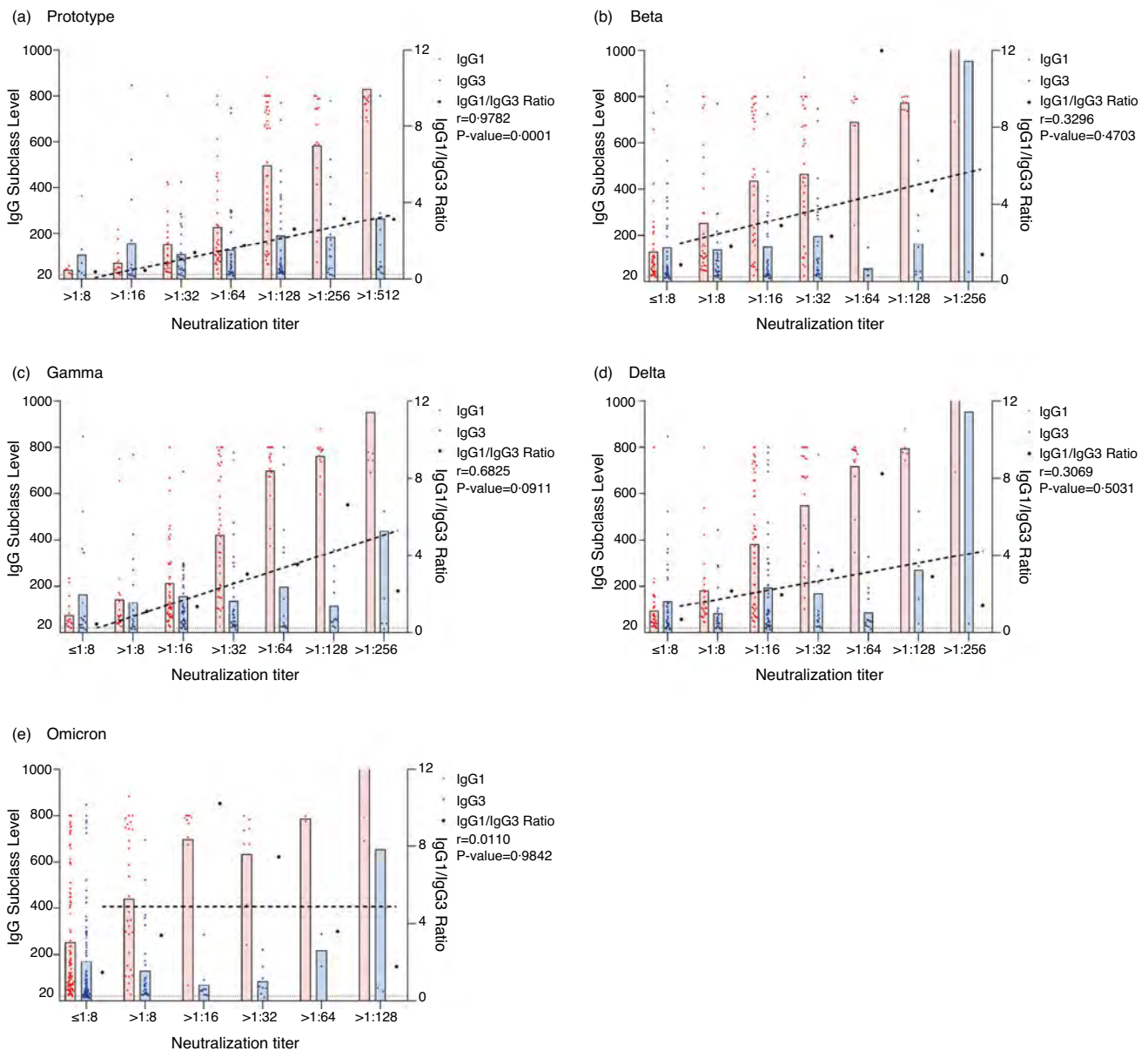


FIGURE 7 The association between IgG subclasses and neutralizing antibodies against the wild-type strain and variants at 3 months after the booster shot. IgG1, IgG3 and IgG1/IgG3 ratio grouped by the titres of neutralizing antibodies against the wild-type strain (a), Beta (b), Gamma (c), Delta (d) and 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.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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CoronaVac

What science proves

7.3. Third dose of CoronaVac maintains high antibody production for six months, study says

A study carried out in China again showed that the third dose of CoronaVac is highly effective in recovering immunity, inducing rapid production of antibodies with prolonged duration. After six months of the booster dose, neutralizing antibody positivity was still over 80%. The work was published in the Journal of Infection and conducted by researchers at the Xiamen University School of Medicine.

In this prospective cohort study, 41 participants received three doses of CoronaVac and had blood samples collected over 180 days after the third dose. Titers of neutralizing antibodies, total anti-RBD antibodies and IgG anti-Spike antibodies were determined to assess the immune response and its duration.

Nine months after the second dose, the production rate of neutralizing antibodies was at 2.44%. It is worth noting that this is a condition common to all Covid-19 vaccines, and is related to the dynamics of SARS-CoV-2. With the third dose, seroconversion reached 100% within

two weeks, remaining constant for another two months, and then slowly starting to decline, dropping to 80.49% within six months.

For total anti-RBD antibodies, the seroconversion rate was 39.02% after nine months of the second dose, peaking at 100% one week after the third dose and remaining that way for six months. The anti-Spike IgG antibody response after the booster dose was similar.

“Neutralizing antibody levels are highly predictive of immune protection. 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 omicron infections or future variants,” the research authors state.

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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 evaluate 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 esti-

mated by an exponential decay model, which increased 2–4 fold compared with those after the second dose⁵ 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 (≤ 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 dose⁵ 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.

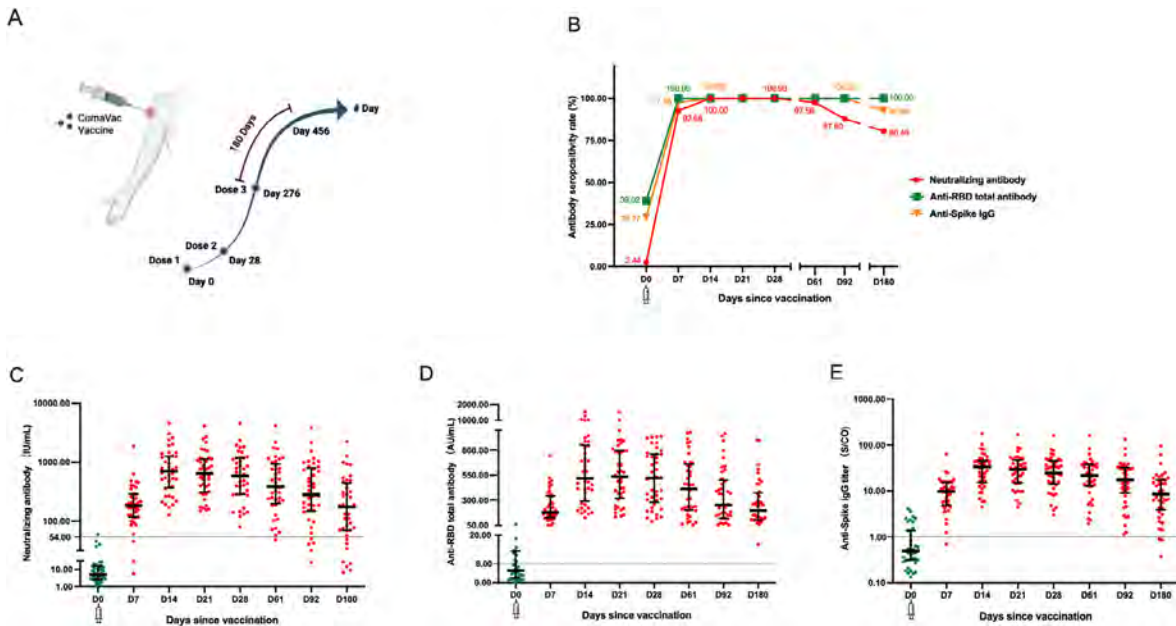


Fig. 1. Anti-SARS-CoV-2 antibody response after the third dose vaccination. A. Schedule of vaccination procedures. B. The seropositive rate changes of antibodies. C-E. The levels of neutralizing antibody (C), anti-RBD total antibody (D) and anti-Spike IgG (E) were measured at 8 serial time points. The antibody-positive judgement threshold is marked with a dotted line.

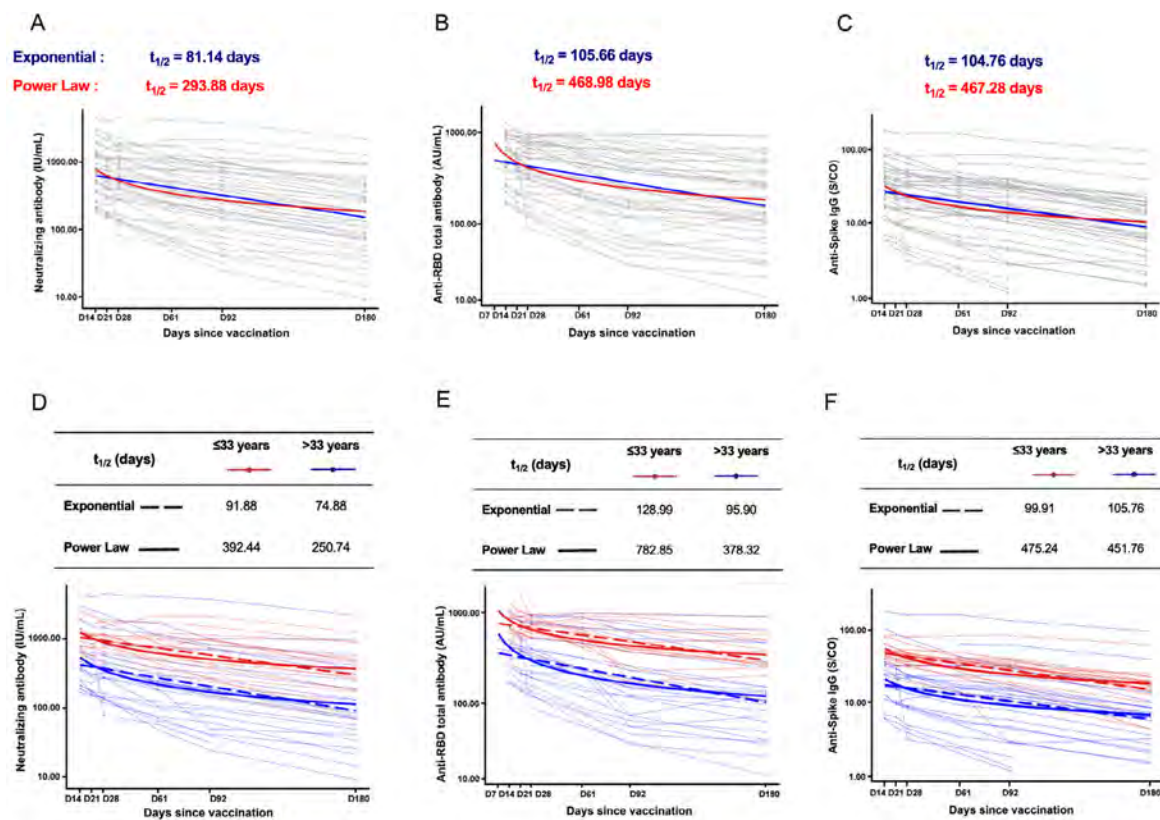


Fig. 2. The exponential and power law model of decay half-lives. A-C: A. Neutralizing antibody; B. Anti-RBD total antibody; C. Anti-Spike IgG. Antibody decay curves and half-lives estimated by an exponential decay model are shown in blue, and the decay curves and half-lives at day 120 estimated by a 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.

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Declaration of Competing Interest

All the authors declare no competing interest in this work.

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7.4. CoronaVac booster dose increases IgG antibodies against omicron by 325 times, study shows

A study carried out in Jiangsu Province, China, showed that the booster dose of CoronaVac induces a potent immune response against the delta and omicron variants of the SARS-CoV-2 virus, increasing the level of specific antibodies produced by 300 to 500 times against these strains. The work was published in the journal *Emerging Microbes & Infections* and conducted by researchers at the Nanjing University School of Medicine.

The researchers selected 100 healthcare workers from the Nanjing University Hospital who received two doses of CoronaVac in February 2021. After nine months, when protection against the SARS-CoV-2 virus naturally reduces, regardless of the vaccine administered, a third dose of CoronaVac was administered to 77 of the subjects. Serum samples were collected at three different times: before the third dose, two weeks later and two months later.

The CoronaVac booster dose activated immunological memory

in all subjects. Before the third dose, the mean titer of anti-RBD IgG antibodies (receptor-binding domain) was 3,278 for the original Wuhan virus, 197 for the delta variant and 44 for omicron. After boosting, the titer increased 17-fold (to 56,760) against the original strain, 577-fold (113,773) against delta, and 325-fold (14,336) against omicron. Two months later, titers remained high, at 82,666, 56,861 and 9,277, respectively.

“Our study highlighted that a booster dose of CoronaVac can provide 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” the authors point out.

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The third dose of CoronVac vaccination induces broad and potent adaptive immune responses that recognize SARS-CoV-2 Delta and Omicron variants

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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 owing 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 fibrin 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 H₂SO₄. 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 (TCID₅₀) 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 1x10³ TCID₅₀ pseudoviruses per well for 1 hour, together with the virus control and cell control wells. The mixture was added to 2 x 10⁴ HEK293T-ACE2 cells (Cat# DD1401-01, Vazyme, Nanjing, China) per well and incubated for 48 hours in 5% CO₂ 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 10⁶ 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 ancestral 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⁺4-1BB⁺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 (94,925,136,363) and 14,336 (11,025,18,641), 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 (57,308, 119,244), 56,861 (45,630, 70,856) and 9,277 (5,903, 14,581), 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 1,009 (562.8-1,808) after the booster, and then dropped to 376 (173.3-814.1) 2 months 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 from different SARS-CoV-2 variants in our cohort was tested. CD4⁺ T cell responses to Alpha and Delta spike were reduced compared to that of 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 CD8⁺ T cells were 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 phenotypic 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.

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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-Šidák 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% pseudovirus neutralization (ID₅₀) 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-Šidák 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.

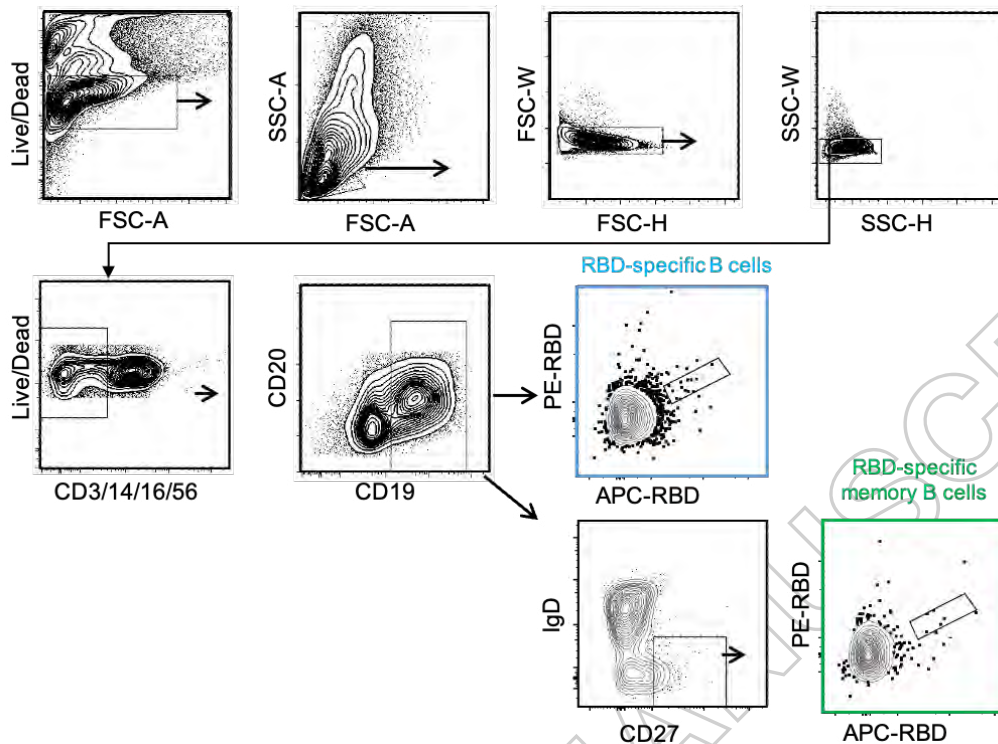
(A) The frequency of cTfh responding to ancestral spike, Alpha spike, Delta spike and Omicron spike (T2 only) of total cTfh cells over time in booster recipients. Dotted lines indicate the lower limit of detection (LOD) for the assay. **(B)** Fold change for the frequency of cTfh cells recognizing Alpha, 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-Šidák 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

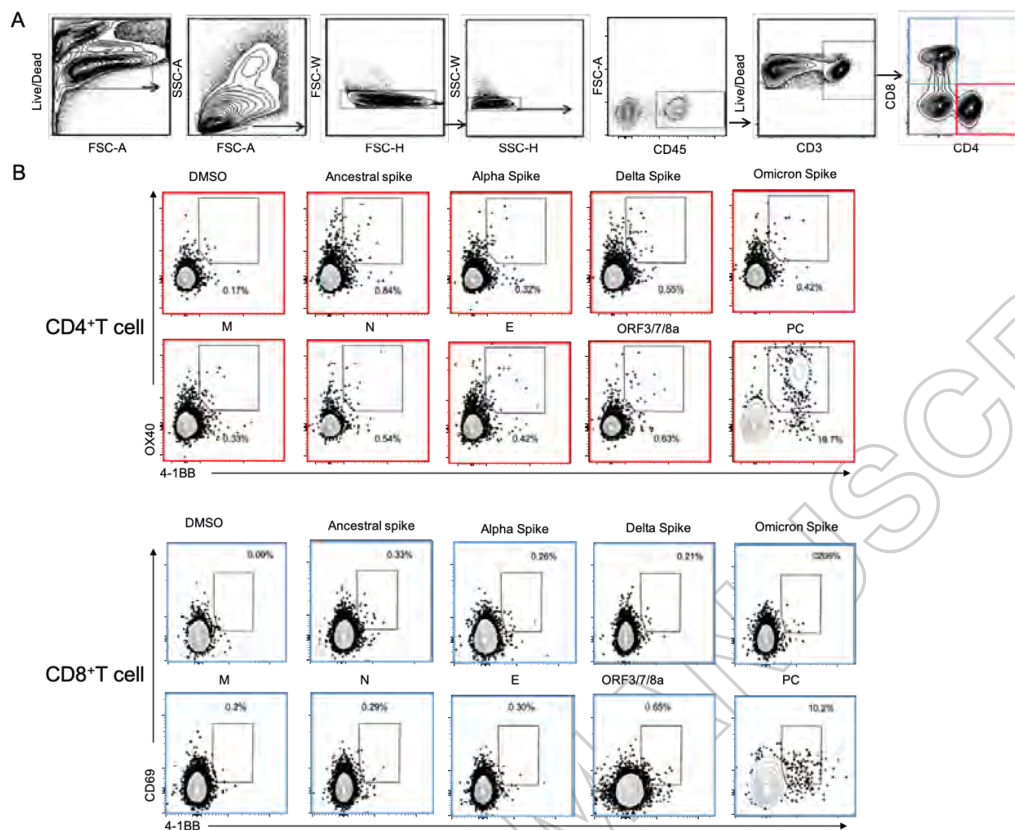
ancestral spike (T0-T2), Alpha spike (T0-T2), Delta spike(T0-T2), and Omicron spike (T2 only) over time in booster recipients. Dotted lines indicated the limit of detection (LOD) for the assay. **(C-D)** Fold change for the frequency of CD4⁺ T cells **(C)** or CD8⁺ T cells **(D)** recognizing Alpha, 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-Šidák 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.

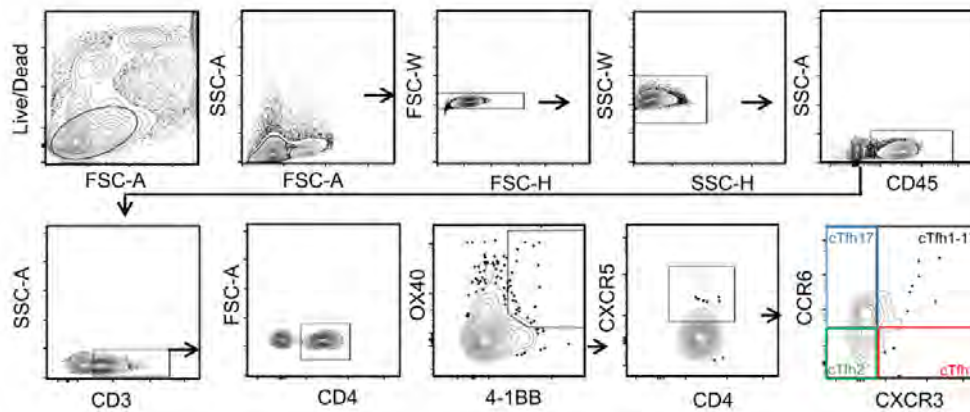
	CoronaVac booster group (n=77)	breakthrough infection group (n=10)
Sex		
Male	32(41.6)	2(20%)
Female	45(58.4)	8(80%)
Age (years)		
Median age (IQR)	35.0(28.3, 40.0)	45.0(44.3, 47.8)
Age group, years		
18-29	26(33.8)	0(0%)
30-39	30(39.0)	0(0%)
40-49	14(18.1)	9(90%)
50-59	7(9.1)	1(10%)
Sample types	serum 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.

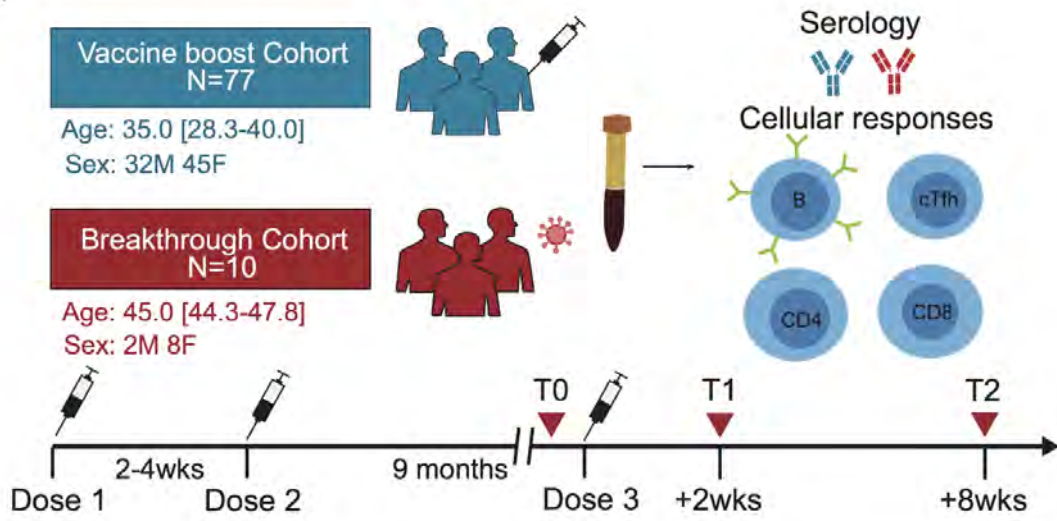


Supplemental Figure 2. Gating strategies to define SARS-CoV-2 specific T cells. (A) Live cells were identified as Live/Dead⁻ and lymphocytes were gated based on forward- and side-scatter. Doublets were then excluded by FSC-W vs. FSC-H and SSC-W vs. SSC-H. Total T cells were identified as CD45⁺CD3⁺, which 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).



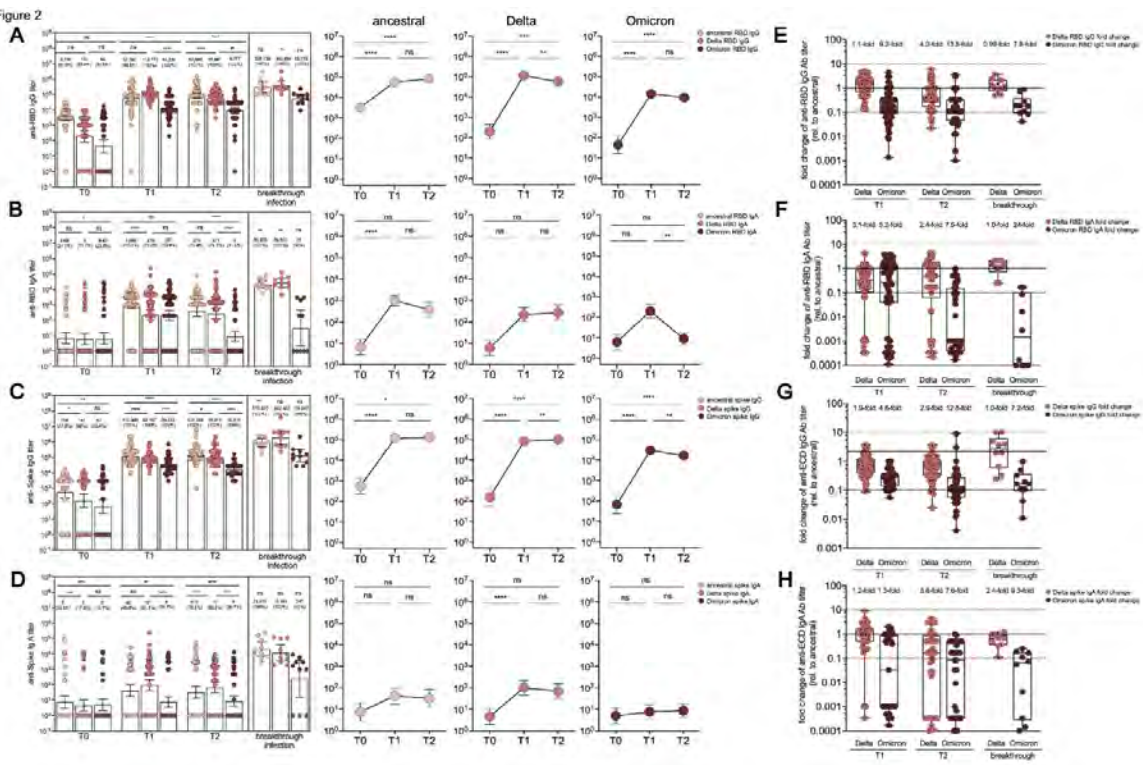
Supplemental Figure 3. Gating strategies to define SARS-CoV-2 spike specific circulating Tfh (cTfh) cells. Live cells were identified as Live/Dead⁻ and lymphocytes were gated based on forward- and side-scatter. Doublets were then excluded by FSC-W vs. FSC-H and SSC-W vs. SSC-H. Total T cells were identified 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



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Figure 2



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Figure 3

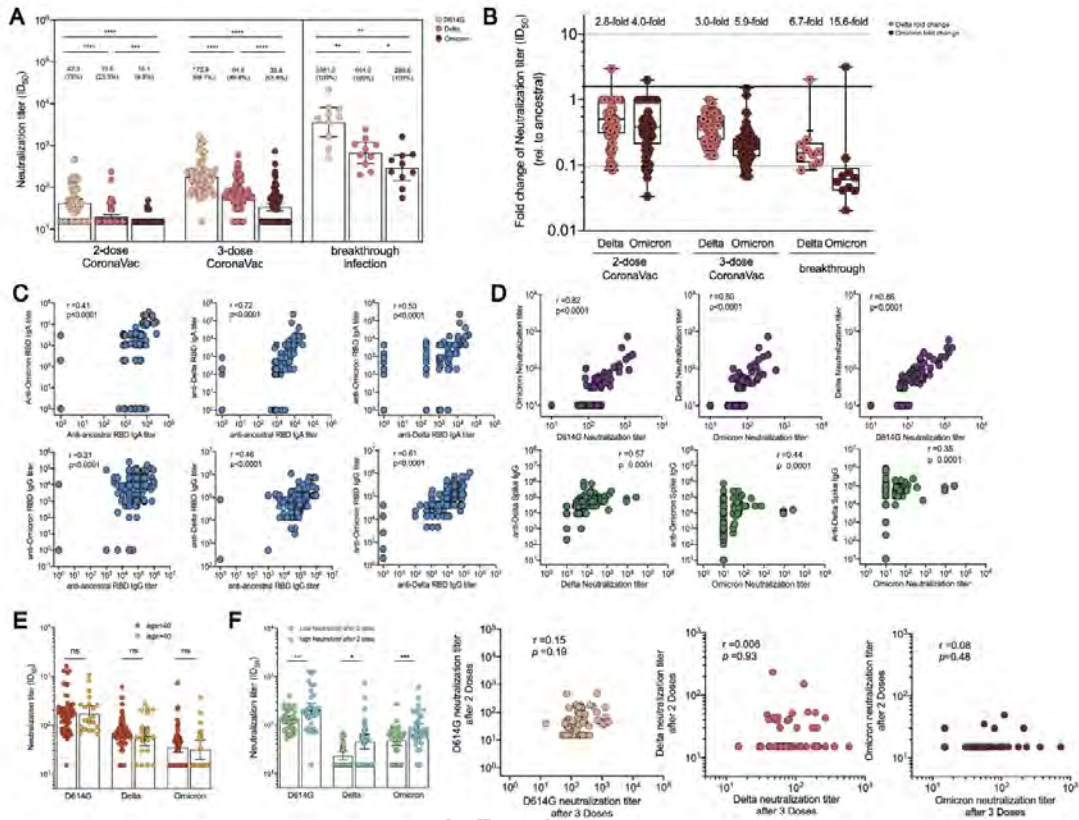
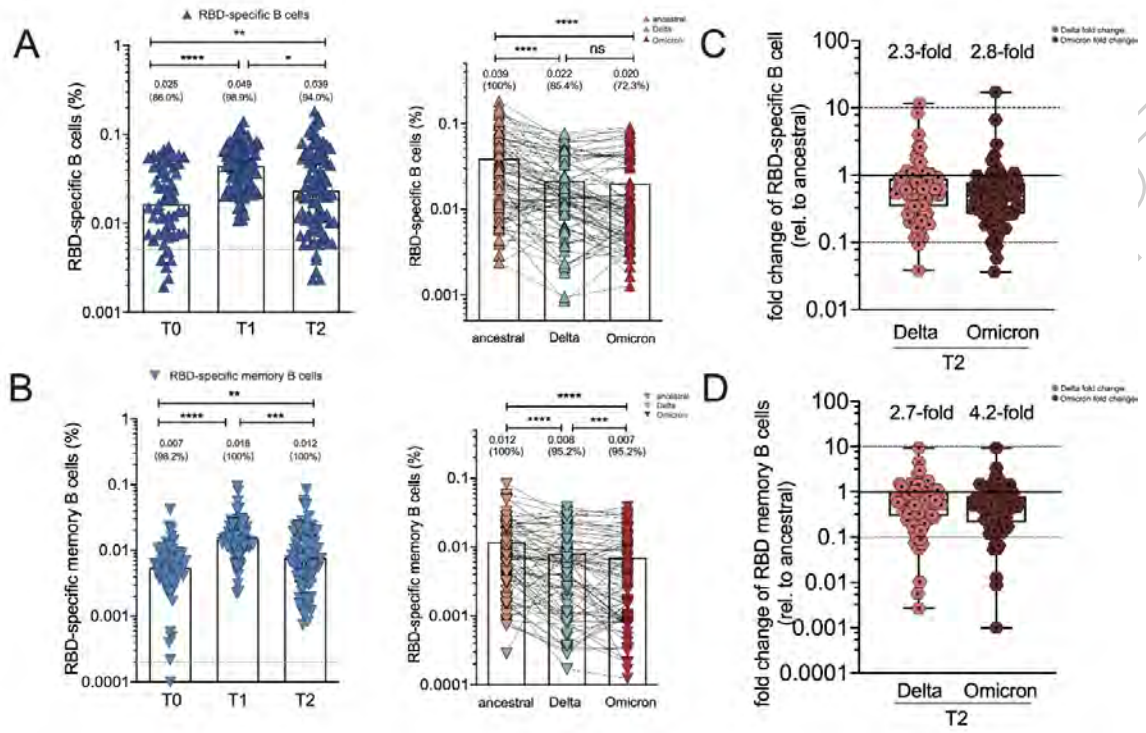


Figure 4



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7.5. Study with 11 million Chileans shows efficacy of CoronaVac booster dose above 85% against severe cases

A study carried out by Chilean researchers and published on the SSRN preprint platform, linked to the scientific journal *The Lancet*, showed that the administration of a booster dose of CoronaVac, a vaccine from Butantan and the Chinese pharmaceutical company Sinovac, showed an efficacy of 78.8% for symptomatic cases, 86.3% for hospitalizations, 92.2% for admissions to Intensive Care Units (ICU) and 86.7% to prevent death related to Covid-19.

This is the largest study ever done on the efficacy of the CoronaVac booster dose and was conducted by researchers from the Chilean Ministry of Health, the School of Medicine of the Pontifical Catholic University of Chile, the Millennium Institute and the Schools of Medicine and Public Health at Harvard University.

The survey was carried out between February and November 2021 and involved 11.2 million people (58% of the population of Chile), aged 16 years and over, who had completed

the primary vaccination schedule with CoronaVac and taken the booster dose of the same immunizing agent. At the time, the delta variant was predominant in Chile.

According to the researchers, the results “suggest that a homologous or heterologous booster dose for individuals with a complete primary vaccination schedule with CoronaVac provides a high level of protection against COVID-19, including severe disease and death.”

The study, published under the title “Effectiveness of Homologous and Heterologous Booster Shots for an Inactivated SARS-CoV-2 Vaccine: A Large-Scale Observational Study”, evaluated the efficacy of induced immune defenses in people who took three doses of CoronaVac in comparison with people not yet vaccinated. Participants with a previous history of Covid-19 infection were excluded.

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Effectiveness of homologous and heterologous booster shots for an inactivated SARS-CoV-2 vaccine: A large-scale observational study

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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.

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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.

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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.

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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*

Characteristic	No.	Col.%	COVID-19		Unvaccinated		Vaccinated					
			No.	Row%	No.	Row%	One dose		Two doses		Three doses	
			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	Cases			Vaccine effectiveness (95% CI)			
	Person-days	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.

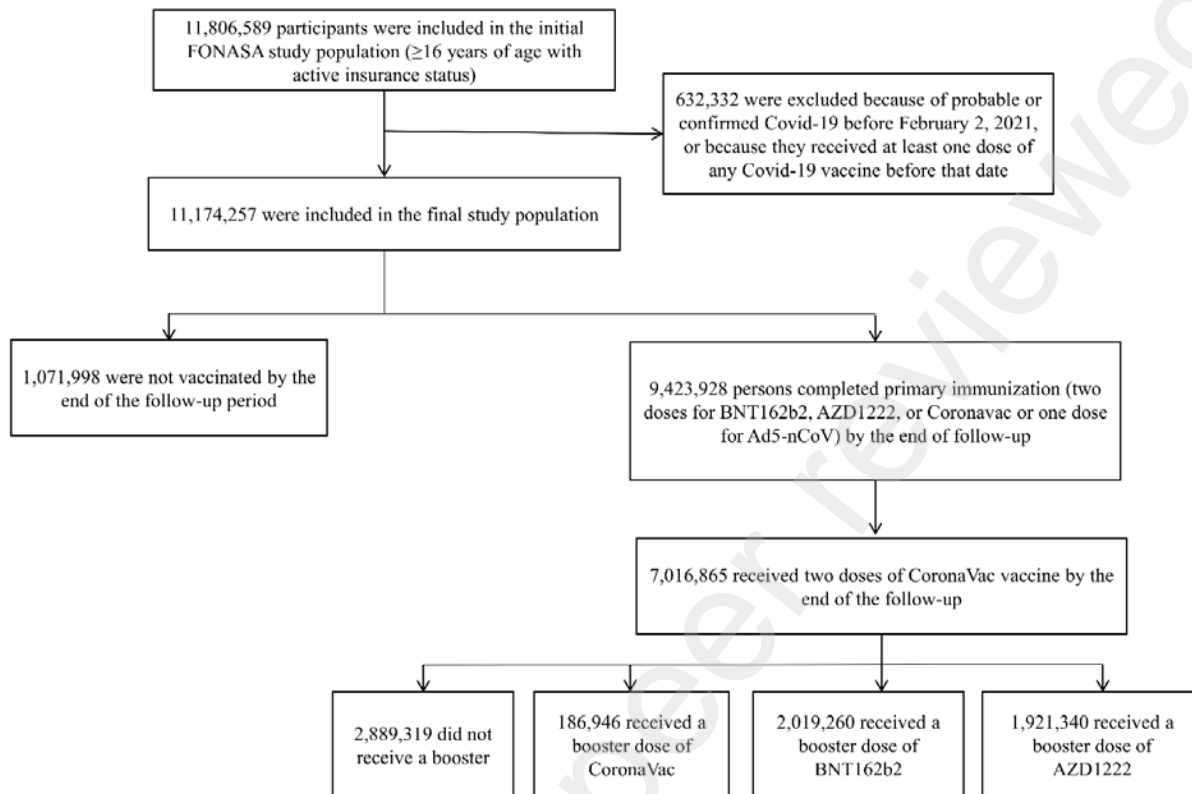


Figure 1. Study participants and cohort eligibility, February 2 to November 10, 2021. Participants were ≥ 16 years of age, affiliated to the Fondo Nacional de Salud (FONASA), the public national healthcare system, and vaccinated with CoronaVac, BNT162b2, AZD1222, or Ad5-nCoV Covid-19 vaccines between 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

What science proves

7.6. Booster dose of CoronaVac administered eight months after the second dose increases up to five times the level of neutralizing antibodies

A research published in The Lancet Infectious Diseases journal showed that the booster dose of CoronaVac, vaccine from Butantan and Sinovac, can increase from three to five times the production of neutralizing antibodies in adults, including elderly with more than 60 years of age. The study was conducted by Chinese researchers from Fudan University, from Sinovac and from the Centers for Disease Control and Prevention of Nanjing and Hebei.

In the first analysis, 271 participants aged from 18 to 59 years immunized with CoronaVac received the booster dose eight months after the second dose, resulting in an increase from three to five times in the titers of neutralizing antibodies (NAb) against the SARS-CoV-2, in comparison with the titers of neutralizing antibodies after the second dose.

A second analysis was made with 303 adults who were 60 or older and also received the booster dose

eight months after the second dose. The results demonstrated that the concentration of neutralizing antibodies rose from 42.9 GMT (or Geometric Medium Titer) on day 28 after the second dose to 158.5 GMT on day 28 after the booster dose - an increase of 3.7 times.

According to the researchers, “our study discovered that a scheme of two doses of CoronaVac generated good immunological memory. The booster dose administered eight months after the second dose had a high efficacy in remembering the specific immune response of SARS-CoV-2, leading to a significant increase in the levels of antibodies”.

Besides, the research indicates that a homologous booster dose (with the same vaccine) can provide a long term immunity and high levels of protection.

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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



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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.

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For the Mandarin translation of the abstract see Online for appendix 1

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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 BNT162b2³⁻⁵ (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-CoV2373¹² (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

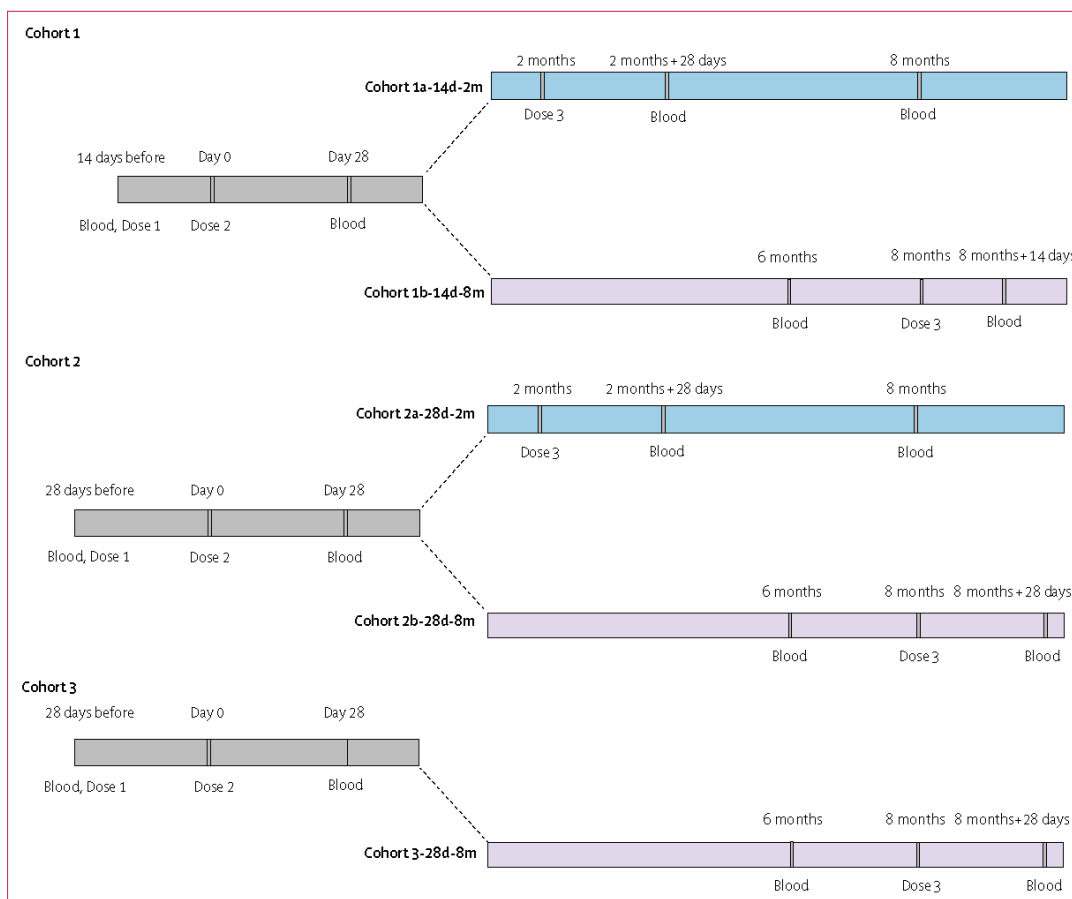


Figure 1: Trial process timeline
Blood–blood sample taken.

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 (TCID₅₀) 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), GMTs 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), GMTs 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). GMTs 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.

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

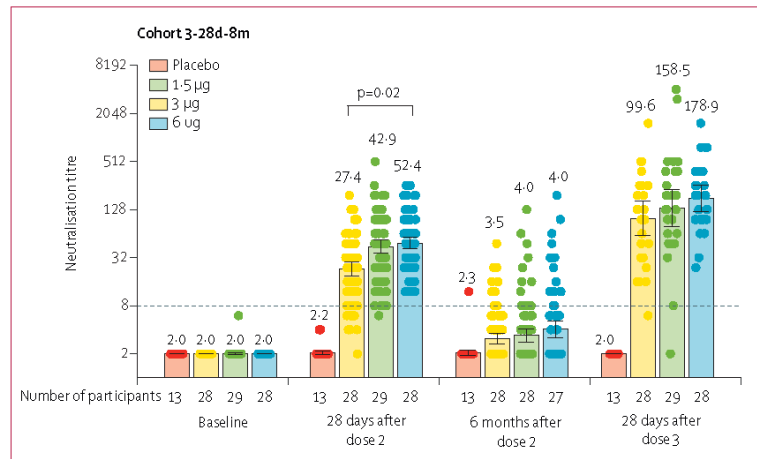


Figure 3: Level of neutralising antibodies to infectious SARS-CoV-2 in adults aged 60 years and older. Dots are reciprocal neutralising antibody titres for individuals in the per-protocol population. Numbers above the bars are GMTs, and the error bars indicate the 95% CI. The dotted horizontal line represents 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.

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.⁵²⁶ 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	1 (2%)	1 (2%)	1 (4%)	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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 (i.e. adverse events related to vaccination).

Table 3- Adverse reactions within 28 days after the third dose

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 vaccines³³ have shown strong short-term immune responses and tolerable reactogenicity. Wanlapakorn and colleagues³⁴ 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 severity⁴¹ 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.⁴⁴ Considering sustained protection of primary immunisation with COVID-19 vaccines against severe outcomes⁴² 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.html>. 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.

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7.7. Booster dose of CoronaVac increases over 12 times the level of antibodies of those that received both doses of the vaccine

Chilean, American and Chinese researchers verified that the booster dose of CoronaVac, a vaccine from Butantan and the Chinese pharmaceutical Sinovac, increases over 12 times the level of antibodies on those that received both doses of the immunizer at least in the previous five months. The results of the study “A booster dose of an inactivated vaccine increases neutralizing antibodies and T cell responses against SARS-CoV-2” were published in the preprint platform medRxiv.

“After the booster dose, the capacity of neutralization increased even more than the one reported two weeks after the second dose. We observed that, four weeks after the booster dose, the neutralizing capacity increased over 12 times in comparison with the response five months after the second dose, and increased more than two times in comparison with the levels registered two weeks after the second dose”, said the researchers from the Millennium Institute of Immunology and Immunotherapy, of the Pontifical Catholic University of Chile; Immunology Institute La Jolla, of the California University in San Diego, from United States; and from Sinovac.

The study had 129 volunteers that received the first dose of CoronaVac

from January to March of 2021, and the second dose with a gap of 28 days. After five months, the participants received the booster dose. The neutralization capacity of the antibodies was evaluated on 77 volunteers.

In adults between 18 and 59 years old, the neutralization capacity of the circulating antibodies reached its maximum four weeks after the booster dose, increasing over 18 times in comparison to the registered levels five months after the second dose, and over four times compared with the registered levels two weeks after the second dose. The seroconversion of this group reached 100% four weeks after the second dose.

In a normal scheme of immunization of two doses with a gap of 28 days, the peak in the neutralization capacity of the antibodies is reached two weeks after the second dose. Among individuals older than 60, that corresponded to 53,2% of the volunteers, the researchers observed that after the booster dose there was an increase of over nine times in the neutralizing capacity in comparison to the response observed five months after the second dose.

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A booster dose of an inactivated vaccine increases neutralizing antibodies and T cell responses against SARS-CoV-2.

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NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.

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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-induced 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 μ g 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: $100 * [\text{OD}_{450\text{nm}} \text{ value of negative control} - \text{OD}_{450\text{nm}} \text{ value of sample}] / [\text{OD}_{450\text{nm}} \text{ of negative control}]$. 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 $39.0 \pm 32.4-47.0$) and more than 2-fold as compared to the two weeks after the second dose (GMU $168.0 \pm 126.8-222.5$) (**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 $918.8 \pm 623.4-1354$) increasing more than 18-fold as compared to five months after the second dose ($48.9 \pm 37.6-63.5$) and more than 4-fold as compared with two weeks after the second dose (GMU $220.2 \pm 150.7-321.7$) (**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 $134.1 \pm 89.2-201.6$), decreasing at four weeks after the second dose (GMU $104.1 \pm 71.8-151.0$), and reaching its minimum at five months after the second dose (GMU $32.4 \pm 25.1-41.8$). In this group, we also observed an increase of more than 9-fold (GMU $300.5 \pm 203.5-443.6$) 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 2nd 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.

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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

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Figures

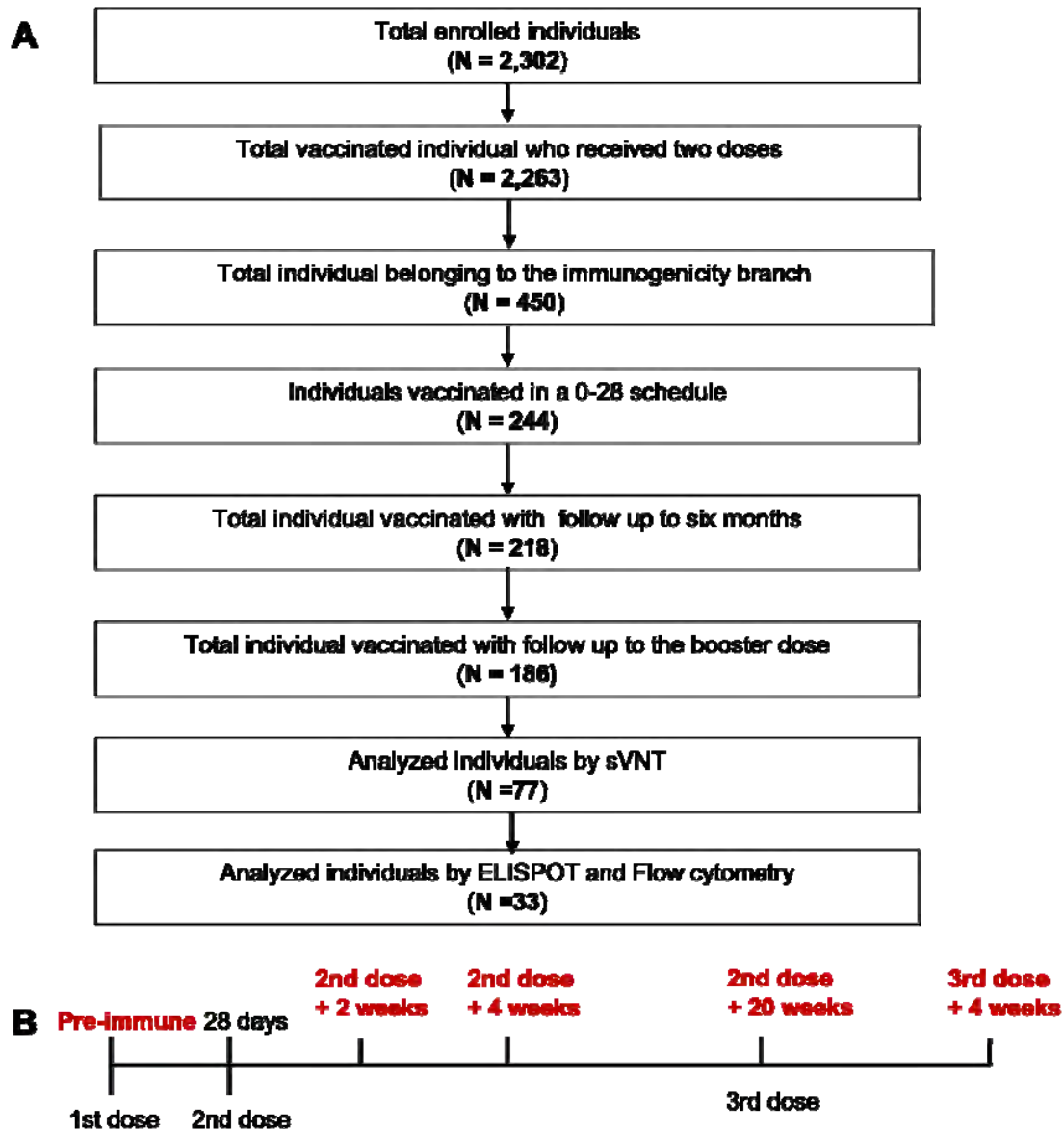


Figure 1: Study profile, enrolled volunteers and cohort included in this study on October 31st, 2021. 77 of the 450 vaccinated individuals belonging to the immunogenicity branch of the clinical trial conducted in Chile were selected of one of the centers of the study (the CL1-Marcoleta) for immunogenicity 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.

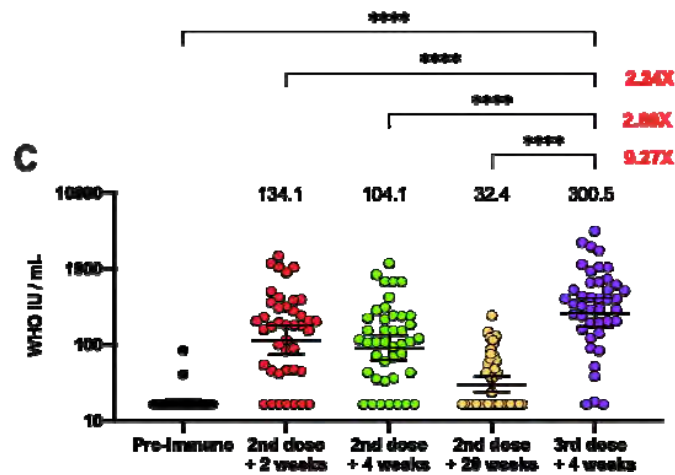
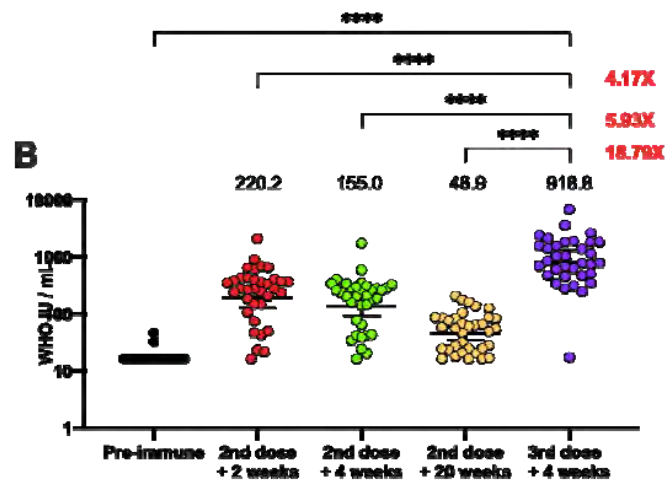
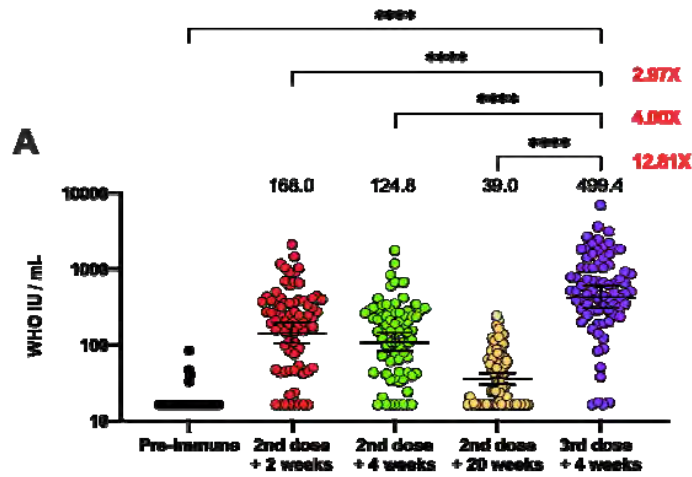


Figure 2: Quantification of circulating antibodies inhibiting the interaction between the S1-RBD and hACE2 in volunteers that received the booster dose twenty weeks after the second dose, in a 0–28-day vaccination schedule.

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.

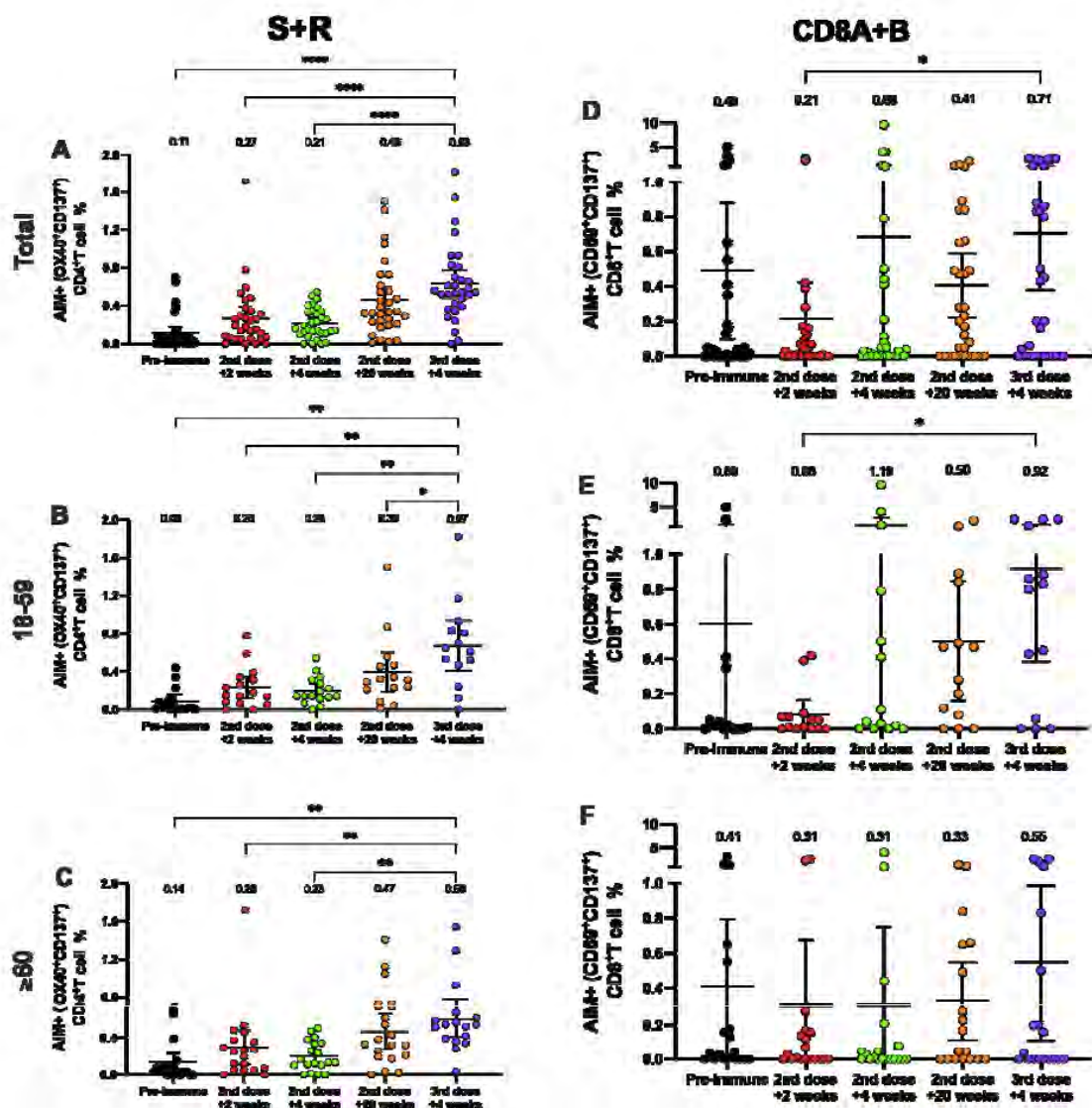


Figure 3: Changes in activation-induced markers (AIMs) expression in T cells through flow cytometry upon stimulation with Mega Pools of peptides derived from SARS-CoV-2 in volunteers immunized with CoronaVac with the booster dose, given twenty weeks after the second dose, in a 0–28-day vaccination schedule. The percentage of activated CD4⁺ (AIM⁺ [OX40⁺, CD137⁺]) and CD8⁺ (AIM⁺ [CD69⁺, CD137⁺]) T cells was determined by flow cytometry, upon

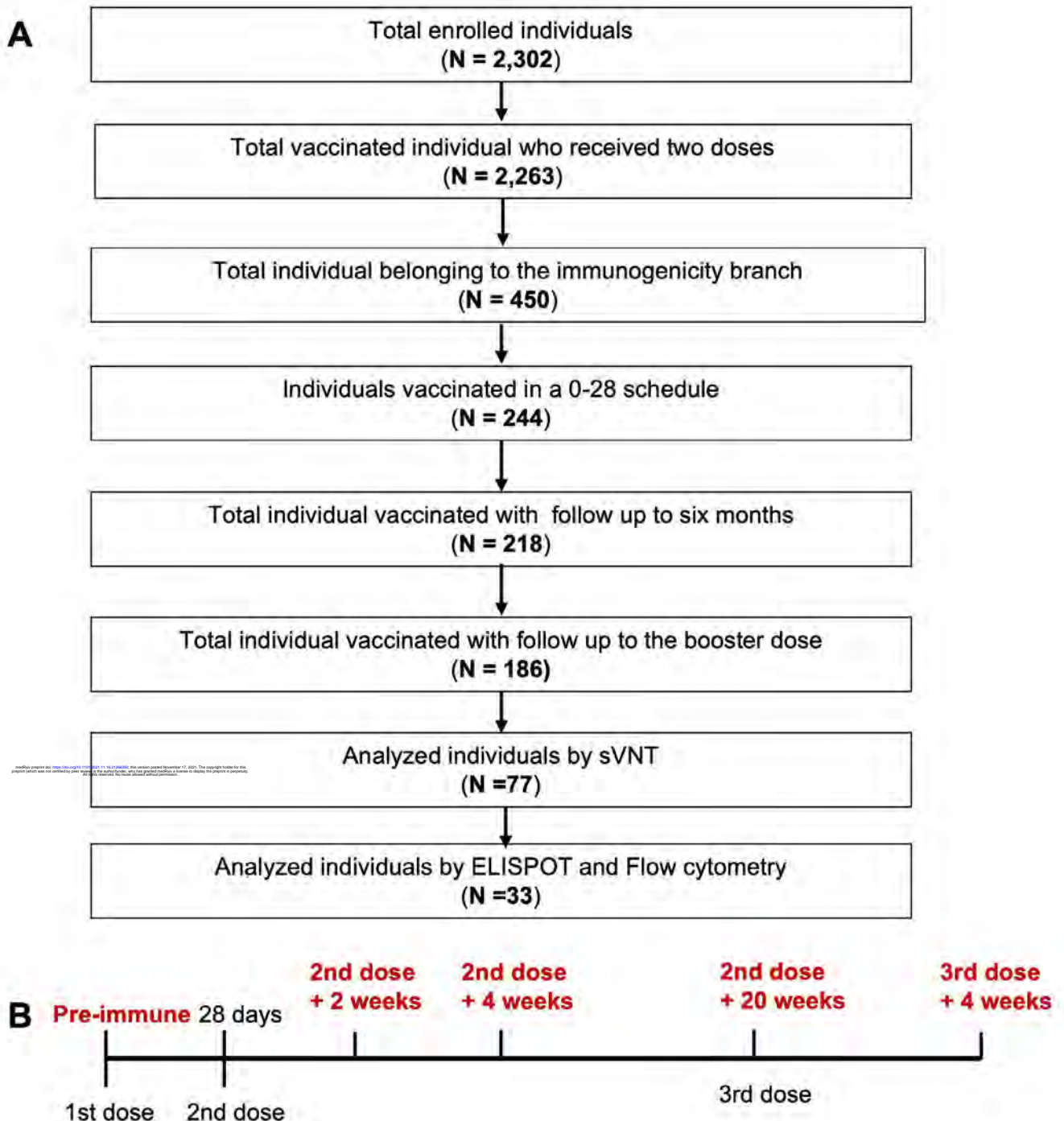
Introduction

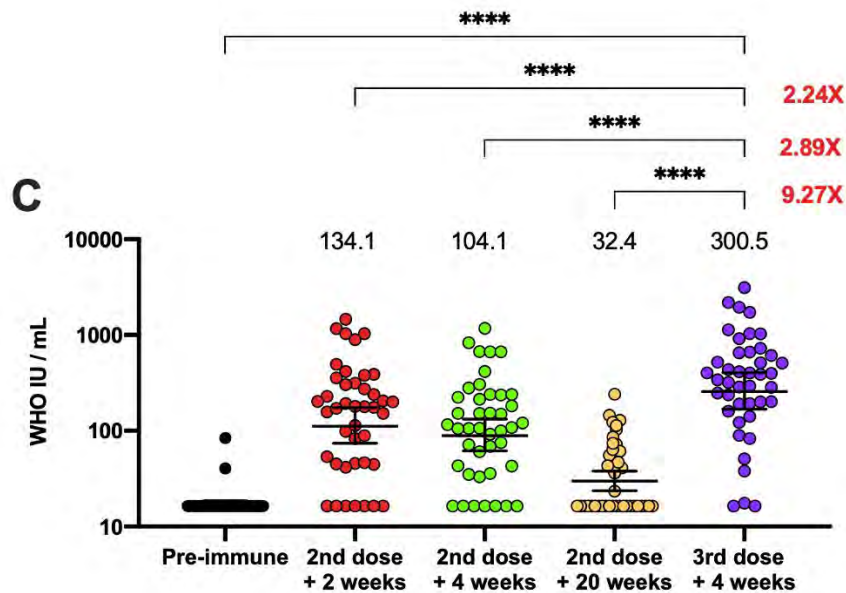
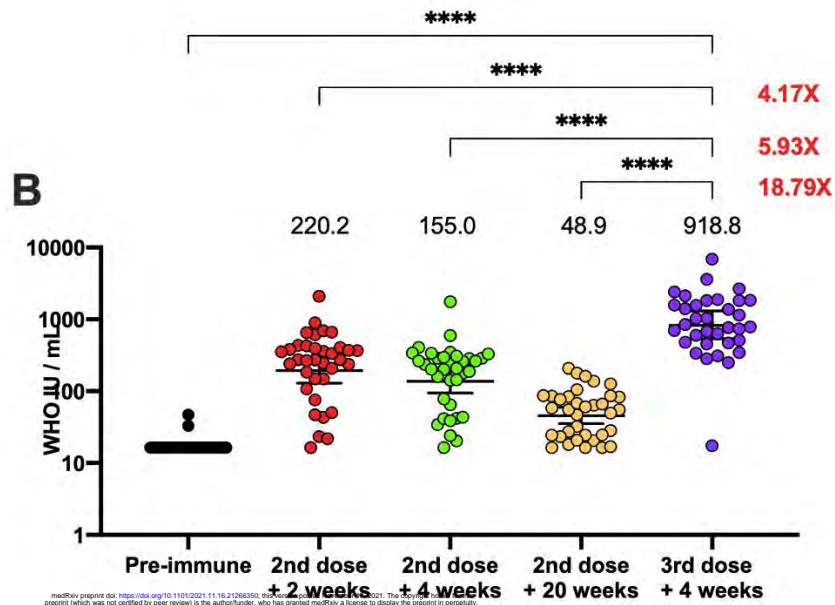
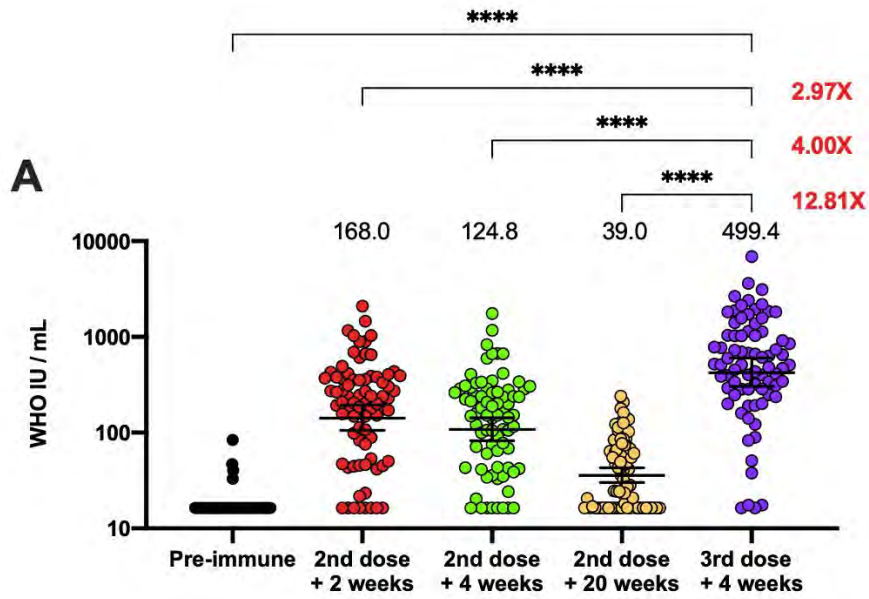
The ongoing pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has promoted the rapid development of safe, immunogenic, and effective vaccines against SARS-CoV-2 to be used by the general population, which have successfully reduced the transmission of the disease burden. 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-induced 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

Table
Table 1: Seropositivity rates, Geometric Median Titer (GMT), and Geometric Median Units (GMU) of circulating neutralizing antibodies against SARS-CoV-2 RBD.

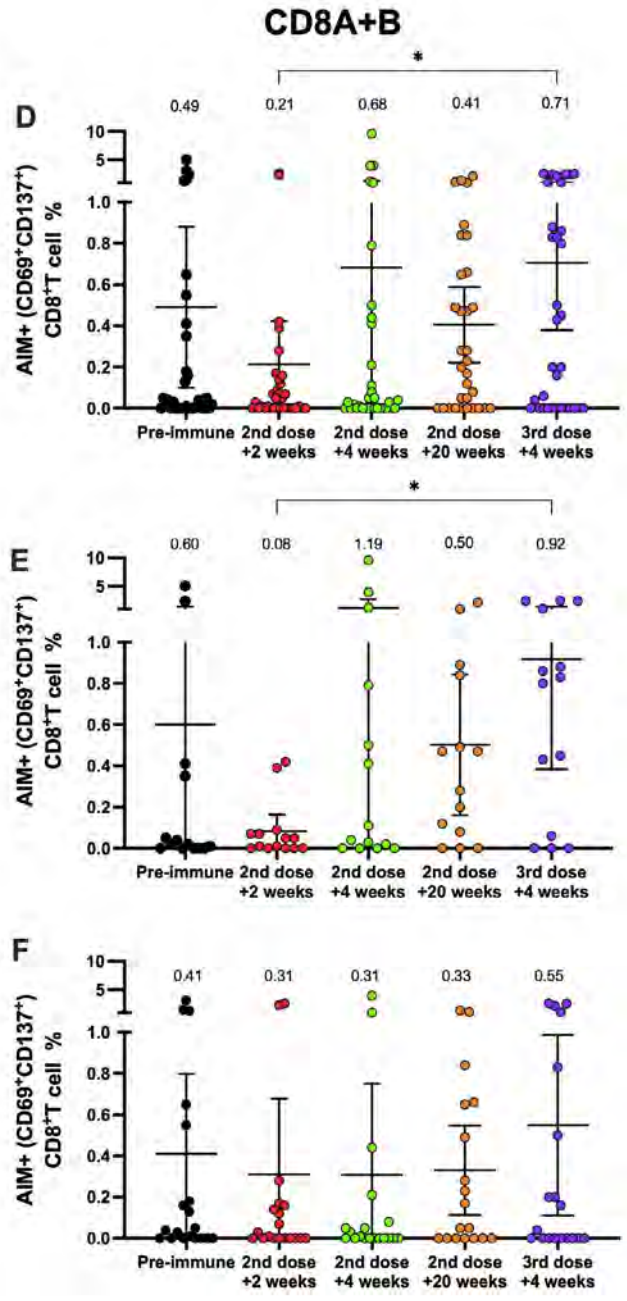
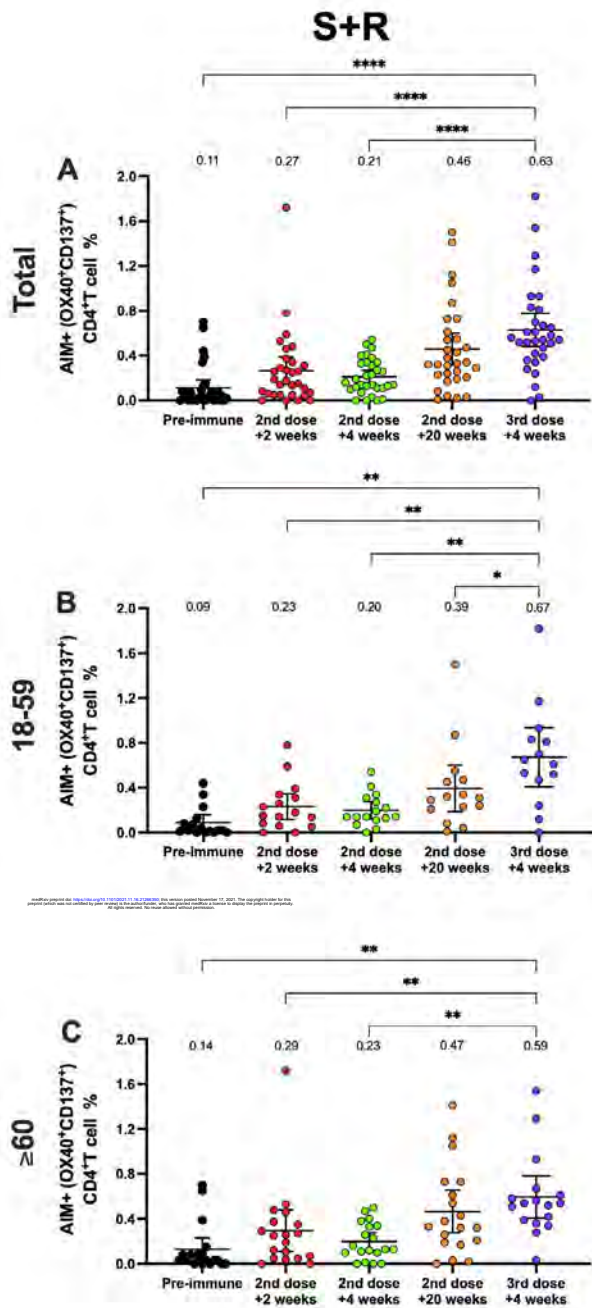
Age group	Indicators	2w after 2nd dose	4w after 2nd dose	5m after 2nd dose	4w after 2nd dose
Total Vaccine	Seropositivity n/N	72/77	73/77	38/77	75/77
	(%)	93.5	94.8	49.4	97.4
	GMU	168.0	124.8	39.0	499.4
	95% CI	126.8-222.5	96.3-161.7	32.4-47.0	370.6-673.0
	GMT	25.8	16.6	3.5	53.0
	95% CI	19.5-34.2	13.1-21.0	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.





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7.8. Booster dose of CoronaVac increases the protection against Covid-19 to 80%, according to Chilean Government

The Health Ministry of Chile announced that the application of a booster dose of CoronaVac, a vaccine from Butantan and the Chinese pharmaceutical Sinovac against Covid-19, increases the efficacy of the immunizer to 80,2%, and expands the protection against hospitalizations from 84% to 88%. The research analyzed the performance of the three vaccines available in the country (CoronaVac, Pfizer and AstraZeneca) in the prevention of cases and hospitalizations, based on the national campaign of vaccination against SARS-CoV-2.

The main conclusion is that the use of a third dose of CoronaVac brings very similar results to the other vaccines, increasing in a considerable way the levels of efficacy against the symptomatic Covid-19. Regarding the protection against general cases, the vaccine of Pfizer-BioNTech increased the indicator from 56% to 90%, and AstraZeneca, from 56% to 93%. And against hospitalization, Pfizer-BioNTech resulted in an increase from 84% to 87% in the protection, and AstraZeneca, from 84% to 96,3%.

The study included 4.785.749 immunized people with the complete scheme of two doses of the vaccine, from whom 2.017.878 received the booster dose beginning on August 11th. Of that group, 1.505.154 received the booster dose of AstraZeneca, 371.592 received the booster dose of Pfizer and 140.132, of CoronaVac. All the participants were older than 16 and didn't have a history of infection by SARS-CoV-2.

According to the infectologist and advisor of the Health Ministry of Chile, Rafael Araos, the study reveals that the decision of applying the additional dose to prevent Covid-19 was right. "The three vaccines that we use as a booster dose on people that were vaccinated with CoronaVac have a super powerful effect"; said the doctor. "The results are robust and suggest that the effect of the booster dose, with any vaccine, has a high efficacy in preventing Covid-19 and hospitalizations."

Published on: 10/07/2021



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



- Evidence suggest that neutralizing antibodies against SARS-CoV-2 induced by vaccines wane over time, which may decrease their effect against Covid-19 and its consequences.
- The longitudinal effectiveness assessments performed by the Chile Ministry of Health showed a sharp decrease 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.

DESIGN AND METHODS

1 ENTREGA DE RESULTADOS
ESTUDIO
DOSIS DE REFUERZO

- We analyzed 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 or 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

1 ENTREGA DE RESULTADOS
ESTUDIO
DOSIS DE REFUERZO

- The effectiveness 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 comorbidities, 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.

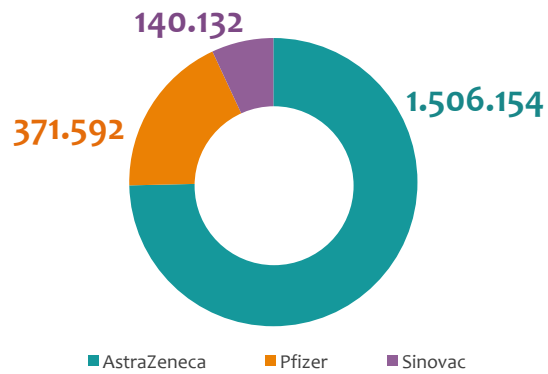
RESULTS | CHARACTERISTICS OF THE COHORT

Characteristic	Covid-19			Vaccinated			p-value	
	N (%)	N (row %)	p-value	Unvaccinated N (row %)	1 dose N (row %)	2 doses N (%)		3 doses N (%)
Total	11.201.635 (100.0)	500.145 (4.5)	-	1.318.288 (11.7087)	719.263 (6.4211)	7.146.296 (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 (66.52)	24.299 (12.1)	
Antofagasta	329.632 (2.9)	10.659 (3.2)		43.639 (13.24)	24.239 (7.35)	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)	
Cóquimbo	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	6.098.579 (37)	184.233 (4.5)		505.660 (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)	666.255 (65.06)	204.959 (19.44)	
Araucanía	683.250 (6.1)	41.357 (6.1)		86.867 (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	584.745 (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 (13.40)	
Magallanes	94.684 (0.85)	4.092 (4.3)		11.295 (11.9)	10.684 (11.28)	53.365 (56.36)	19.370 (20.46)	

RESULTS



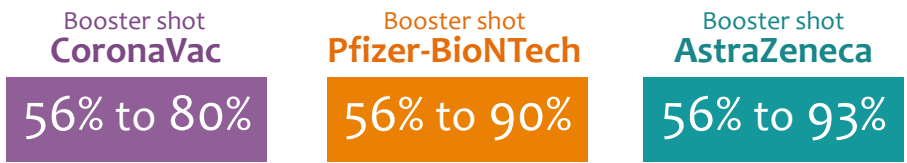
4.785.749 people immunized with CoronaVac were included.
2.017.878 received one booster shot.



RESULTS



INCREASED EFFECTIVENESS AGAINST COVID-19
 14 DAYS AFTER THE BOOSTER SHOT



RESULTS

1 ENTREGA DE RESULTADOS
ESTUDIO
DOSIS DE REFUERZO

INCREASED EFFECTIVENESS AGAINST HOSPITALIZATION

14 DAYS AFTER THE BOOSTER SHOT

Booster shot
CoronaVac

84% to 88%

Booster shot
Pfizer-BioNTech

84% to 87%

Booster shot
AstraZeneca

84% to 96%

9

CONCLUSION

1 ENTREGA DE RESULTADOS
ESTUDIO
DOSIS DE REFUERZO

- The three vaccines used as a booster notably **increased the effectiveness against Covid-19 and related hospitalizations.**
- These results support the decision to initiate a boosting program among people immunized with inactivated vaccines.

10



8 ■ Rare adverse effects

8.1. CoronaVac presents adverse events in less than 1% of doses applied in adults in Brazil and is highly safe for vaccinees

CoronaVac, a vaccine developed by Instituto Butantan in partnership with the Chinese pharmaceutical company Sinovac, had adverse events in less than 1% of the doses applied in people over 18 years old in Brazil. The data from the Institute's Pharmacovigilance are another indicator that the vaccine is highly safe and very non-reactogenic.

"CoronaVac has a very good safety profile and we saw this in the proportion between the number of people vaccinated and the total number of adverse events reported," the scientific researcher and qualified person for Pharmacovigilance at Instituto Butantan, Vera Gattás, explains.

The real-life data reinforces studies carried out with CoronaVac, which indicate that the vaccine is highly safe for adults.

"In numbers, this means that in about 130 million doses administered in the country, there were around 1,000 reported cases of serious adverse events and 7,000 non-serious adverse events, both of which do not reach 1% of the doses applied," Vera informs.

Studies with children over 3 years of age also showed good safety results. The most common reactions were local, such as pain, swelling

and redness at the application site. "We also found that the benefits of vaccination with CoronaVac far outweigh its risks of adverse events," reiterates the Pharmacovigilance leader of Butantan.

Difference between adverse reaction and event

"Every adverse reaction is an adverse event, but not every adverse event is an adverse reaction, and it is up to Pharmacovigilance to make this distinction," Vera explains.

Adverse events are any occurrences that occur after the use of a drug, serum or vaccine, which may or may not be related to the product used.

Adverse reactions are any symptoms or harmful events that occur after the use of the drug, serum or vaccine, with some possibility of having been caused by the use of any of these products.

Among the adverse events reported to Butantan's Pharmacovigilance, most were classified as mild (grade 1) or moderate (grade 2). These are expected events such as pain and pain at the injection site, fever and body pain that resolve within the first few days after administration.

How is pharmacovigilance performed?

The monitoring of adverse event reports is the basis of pharmacovigilance, responsible for continuing the work of verifying the safety, efficacy and quality of vaccines after reaching the population. This monitoring continues to be carried out by the manufacturer after immunization as a way of preventing and detecting changes in the safety profile and in the occurrence and frequency of adverse events.

At Butantan, the monitoring of CoronaVac and other vaccines, medicines, antitoxins and antivenoms is carried out by the Pharmacovigilance team. Notifications arrive through Customer Service (see contacts below) and through the official communication channels of Instituto Butantan.

“We are always alert and ready to receive information that may indicate adverse effects of vaccination or other medicines produced at Butantan. We have several communication channels from which we accumulate data and make an overview that aims to monitor the products manufactured in order to provide information

for the evaluation of the safety, efficacy and quality of the product”, she details.

According to Vera, it is not always clear to the population that Pharmacovigilance maintains the safety, efficacy and quality assurance of the vaccines. And that, in case of any symptoms after vaccination, they must be reported in order to keep control.

“If, after the Pharmacovigilance analysis, it is shown that the report is not necessarily related to the use of the vaccine, it is still important to report it so that the information can be sent for analysis by the technical areas,” the researcher concludes.

How to contact the Pharmacovigilance of Butantan

Customer Service (Serviço de Atendimento ao Consumidor):

Phone: **0800 701 2850**

Email: **sac@butantan.gov.br**

Pharmacovigilance email:

farmacovigilancia@butantan.gov.br

8.2. CoronaVac is safe and has fewer serious adverse effects, Malaysia study shows

A study carried out in Malaysia once again demonstrated the high safety profile of CoronaVac and the low occurrence of adverse reactions after immunization. Among the 9.3 million people who took the vaccine in the country, no serious thrombotic adverse events were recorded. The work was published in the journal *Vaccine* and conducted by researchers from the Malaysian National Institute of Health, the National Pharmaceutical Regulatory Agency and seven hospitals in the country.

By the end of August 2021, 35.2 million doses of Covid-19 vaccine had been administered to more than 20 million people in Malaysia, with CoronaVac being the most used immunizing agent with 17 million doses (48% of the total), followed by Pfizer (44%) and AstraZeneca (8%). About 78% of the 9.3 million individuals vaccinated with CoronaVac completed both doses of the vaccine.

CoronaVac was not associated with any serious thrombotic adverse effects, but was associated with a small increase in the risk of arrhythmia. In individuals vaccinated with a messenger RNA immunizing agent, there was a slight increase in the risk of venous thromboembolism, arrhythmia and seizure. In those who received

the viral vector vaccine, there was a significant increased risk of thrombocytopenia and venous thromboembolism.

However, the researchers did not record risk of myocarditis, pericarditis, Bell's palsy, stroke and myocardial infarction in the 21 days after administration of any of the vaccines. The absolute number of adverse events was low for all vaccines. "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," the authors say.

CoronaVac is safe in patients at increased risk for thrombosis

Research conducted by the School of Medicine of the University of São Paulo (FMUSP) with individuals at high risk of thrombosis, with an autoimmune disease called antiphospholipid syndrome, showed that CoronaVac is a safe vaccine for this group, not influencing the disease. In addition, the immunizing agent was highly immunogenic, inducing antibody production in more than 80% of patients.

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Risk of serious adverse events after the BNT162b2, CoronaVac, and ChAdOx1 vaccines in Malaysia: A self-controlled case series study

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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.

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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

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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.

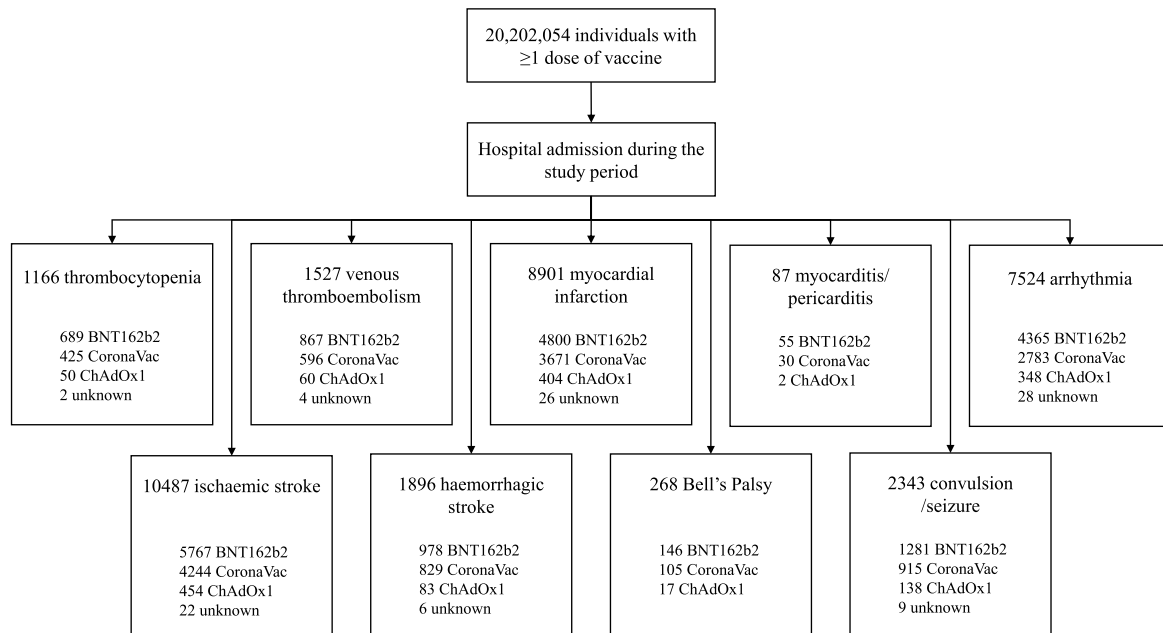


Fig. 1. Flow diagram of study population. The study period is from 1 February 2021 to 30 September 2021. Unknown refers to those whose vaccine type was not available.

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 consid-

ered 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

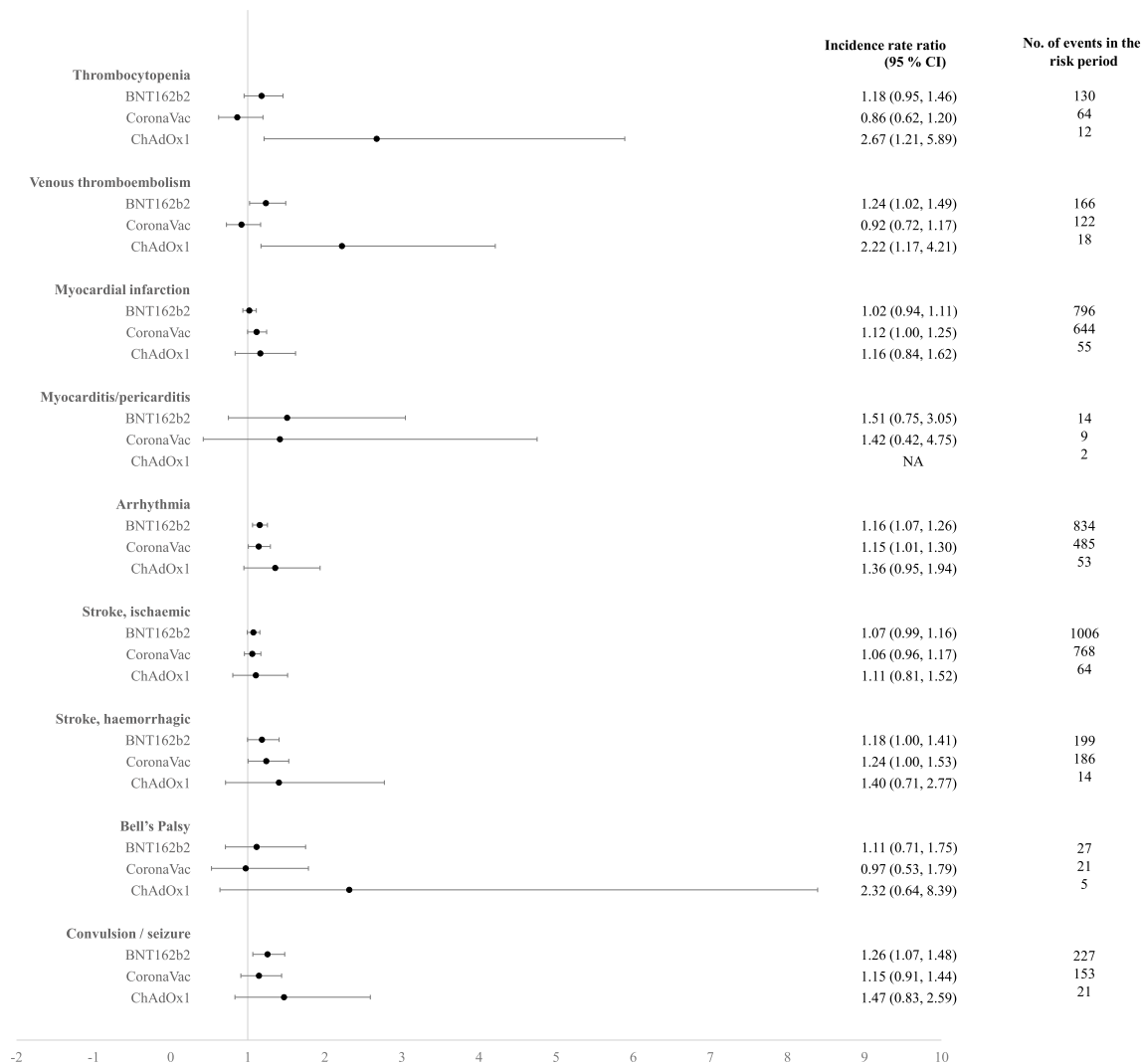


Fig. 2. Incidence rate ratios and 95% confidence intervals of outcome events in the 21-day risk period after either dose of BNT162b2, CoronaVac, and ChAdOx1 vaccines compared with outcome events in the control period, adjusted for calendar month between 1 February 2021 to 30 September 2021. Abbreviation: 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 thrombocy-

topenia, 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.

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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 Nadzroh Ibrahim:** Investigation, Writing – review & editing. **Fatihah Mahmud:** Investigation, Writing – review & editing. **Sheamini Sivasampu:** . **Kalaarasu M Peeri-asamy:** 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.

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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>.

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8.3. CoronaVac has 83% less chance of causing adverse effects than the messenger RNA vaccines

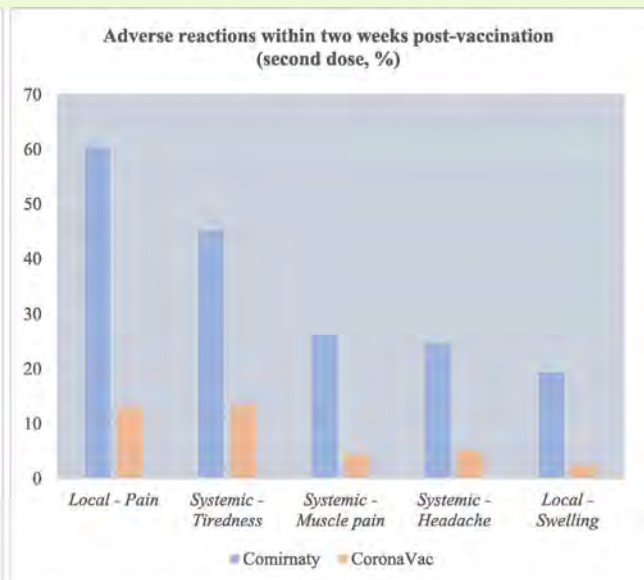
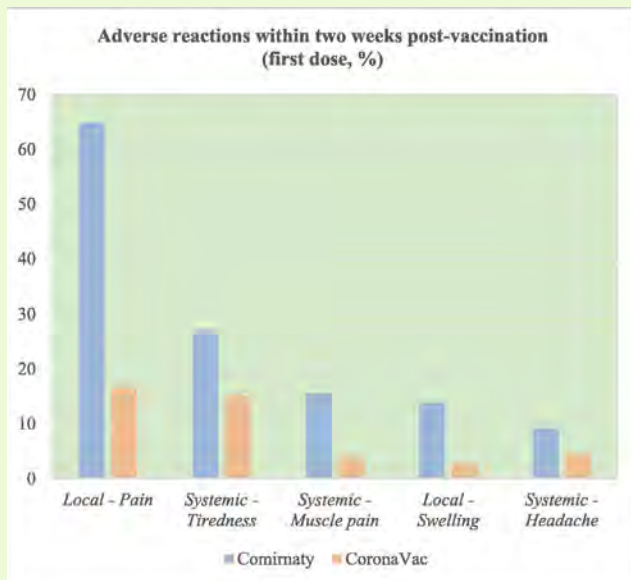
A study published in the journal Vaccines showed that those that received CoronaVac, a vaccine from Butantan and the Chinese pharmaceutical Sinovac, have 83% less chance of experiencing adverse reactions than those who received vaccines made with messenger RNA (mRNA). The research was conducted between February and July of 2021 by scientists of the Health Department from Hong Kong, of the Technology and Science Park of Hong Kong and the University of London.

“The adjusted analysis suggests that, in comparison to Comirnaty (the official name of the vaccine from Pfizer), CoronaVac is associated with 83% less chance of causing any adverse reaction and 76% less chance of causing systemic adverse reactions”, described the study.

The scientists recruited 1,129

individuals that received CoronaVac, with an average age of 46 years, and 969 people that received the mRNA vaccine from Pfizer, with an average age of 43 years. The volunteers were monitored for 14 days after each dose, a period of time when each of them answered a questionnaire about the adverse reactions caused by the vaccination.

During the monitoring period, 82,7% of the participants that received the immunizer from Pfizer reported adverse events, while 48,1% of those vaccinated with CoronaVac reported some kind of reaction. The most common symptoms after the first and second dose for both of the vaccines were pain and swelling in the area of the injection, fatigue, muscle ache and headache. The graphic below compares the percentage of reactions between both vaccines.



CoronaVacs safety was already proven through other studies

The research confirms the findings of other articles already published, such as clinical trials from Turkey and from China, which demonstrated that CoronaVac may cause adverse events at only 18,9% to 33% of the individuals, presenting a high safety profile.

While the clinical trial of phase 3 of the Cominarty vaccine demonstrated that about 80% of the volunteers presented adverse reactions after receiving the immunizer.

According to scientists, studies have already shown that the reactogenicity is one of the factors that have influence in the population's decision about getting vaccinated or not. Studies that clarify the possible adverse effects and testifies the safety of the vaccines are important to increase public trust in vaccines.

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Self-reported reactogenicity of CoronaVac (Sinovac) compared with Comirnaty (Pfizer-BioNTech): A prospective cohort study with intensive monitoring

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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.

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1. Introduction

CoronaVac (Sinovac) Covid-19 vaccine, an inactivated virus vaccine, has been approved for emergency use by the World Health

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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	Standardized mean difference	
n	1129	969		
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-

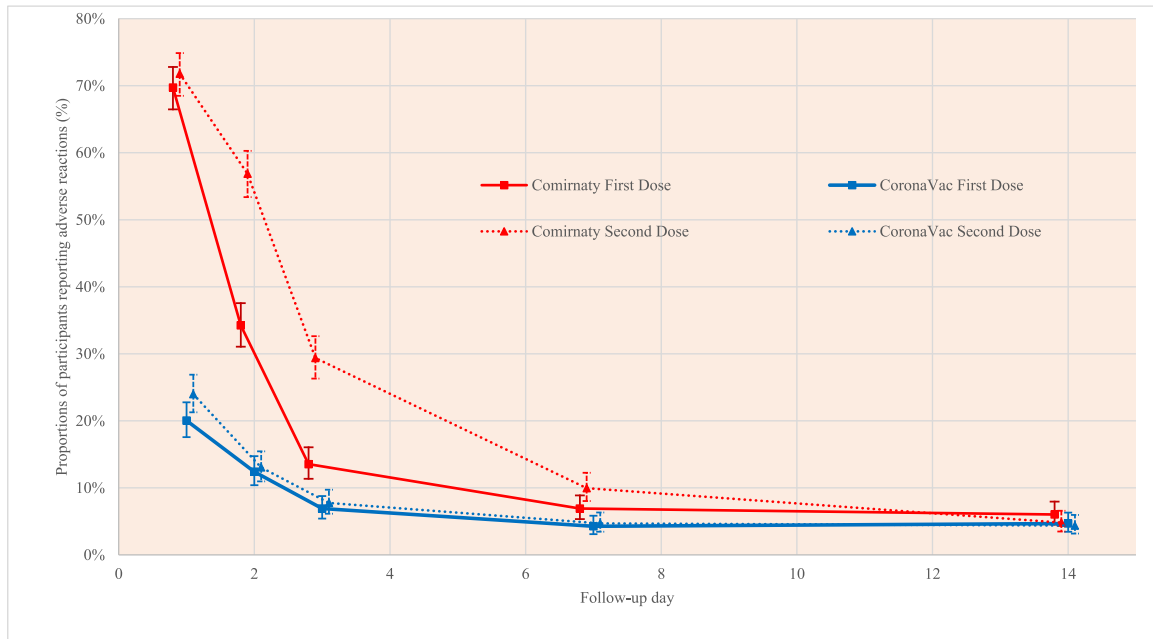


Fig. 1. Proportions (with 95% confidence intervals) of self-reported adverse reactions by vaccine type and dose (first versus second). Sample size varies across timepoints with different retention rate on different follow-up days.

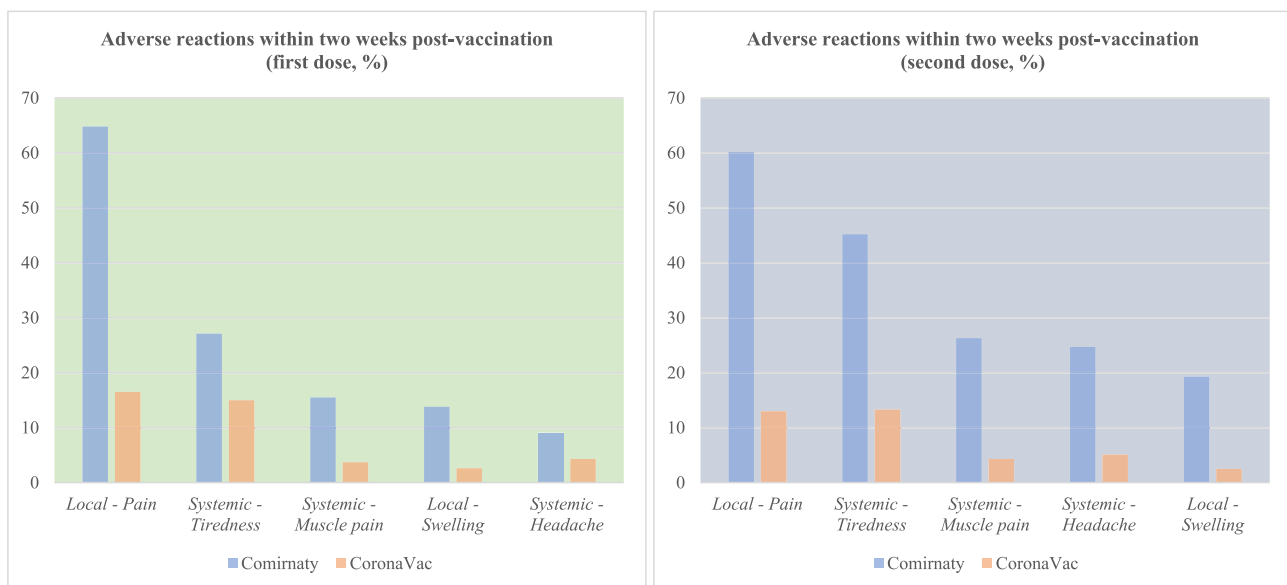


Fig. 2. Proportions of participants reporting specific adverse reactions two weeks post-vaccination.

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 hospi-

talized. 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 reactivity 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.

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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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2022.01.062>.

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CoronaVac

What science proves

8.4. CoronaVac is the vaccine with less adverse effects among the ones used in Brazil

A study published in the scientific journal *The Lancet Infectious Diseases* revealed that CoronaVac, a vaccine from Butantan and the Chinese pharmaceutical Sinovac against Covid-19, causes adverse reactions in only 29% to 33% of the vaccinated, and all very mild (such as pain in the arm and temporary fatigue). This is a great indicator that testifies the high safety profile of the immunizer, and one of the smallest index of adverse effects among all the approved vaccines until this moment for emergency use by the World Health Organization.

The research was made by scientists of the Centers for Disease Control and Prevention from the provinces of Hangzhou, Nanjing and Jiangsu, in China, scientists of the Chinese Academy of Science and researchers from Sinovac, with 744 volunteers that took part in the clinical trials of phase 1 and 2 of CoronaVac. During phase 1, 29% of the volunteers reported to have felt adverse reactions, mainly pain in the area of the injection and fatigue, in the period of 14 days after receiving the vaccine. During phase 2, only 33%

of the volunteers reported adverse effects. Less than 5% of the volunteers on both phases had symptoms like fever, headache or nausea.

In Brazil, data about the safety of Butantan's vaccine were obtained in clinical trials of phase 3 with 9 thousand volunteers in 2020. The adverse manifestations were very mild and did not require medical attention. In Project S, a clinical trial conducted by Butantan in the city of Serrana, 54.882 doses were administered in the adult population and there was no report of severe adverse reactions related to the vaccination. During the application of the first dose of the immunizer in Serrana, there were 4,4% of adverse reactions reports and only 0,02% of them were considered level 3 (myalgia and headache), because it interfered in the daily activities. After the second dose there were only 0,2% reports of adverse effects, none of them considered level 3 or superior. Another indicator that testifies the safety of CoronaVac is that, up to this date, the area of Pharmacovigilance of Butantan did

not receive any report of thrombosis associated with the vaccination – one of the adverse effects that were already reported in other vaccines against Covid-19.

Those results contrast with the conclusions observed in studies with the other vaccines against Covid-19 – although it is not possible to compare directly the results of the research, since the studied groups are different, and so are the methodologies of analysis. Between 70% and 75% of the north americans that received vaccines made with the technology of messenger RNA (mRNA) reported having adverse effects, a percentage that increased from 86% to 88% among british patients that received the vaccine of AstraZeneca/Oxford, made with viral vector technology. In the case of the Janssen vaccine, also of viral vector, between 35% and 62% of the interviewed people related adverse reactions.

The technology used in the CoronaVac, made with inactivated viruses, is one of the most studied

and safe in the world. The virus is replicated and, afterwards, killed. Therefore, it is not capable of multiplying into the body and causing the disease, but can initiate the production of antibodies and induce immunological response.

Vaccine made with messenger RNA technology (mRNA)

A study published in the American Association of Medicine journal in April of 2021, about the perception of the adverse effects of the vaccines from the american pharmaceuticals Pfizer or Moderna, produced with the technology of messenger RNA (mRNA), was made with 3,6 million of north americans that received the first dose and 1,9 million that received the second dose. The majority of the participants reported having experienced pain in the area of the injection (70% of those that received the first dose, and 75% of those that received the second) or systemic reaction (50% after the first dose, and 69,4% after the second dose) during the first

seven days after the vaccination. The most frequent reactions after the first dose of the vaccine were pain in the area of the injection (67,8%), fatigue (30,9%), headache (25,9%) and myalgia (19,4%). The report of adverse effects was higher after the second dose for both vaccines, especially for reactions as fatigue (53,9%), headache (46,7%), myalgia (44%), shivers (31,3%), fever (29,5%) and joint pain (25,6%).

Vaccines made with viral vectors

A study published in The Lancet in November of 2020 analyzed the perception of adverse effects on 560 adults that received the vaccine made by the anglo-swedish pharmaceutical AstraZeneca and by researchers of the Oxford University. Among those that received two doses of the vaccine, after the first dose local reactions were reported in 88% of the participants in the group of 18 to 55 years of age, 73% in the group of 56 to 69 years of age, and 61% in the group of 70 years

or older. Systemic reactions were reported in 86% of the participants in the group of 18 to 55 years of age, 77% in the group of 56 to 69 years of age, and 65% in the group of 70 years of age or more. Fatigue, headache, fever and myalgia were the most common systemic adverse reactions reported.

Besides that, the Center for Disease Control and Prevention from the United States conducted a data survey in August of 2021 with 3.356 North Americans that received the single dose of the Janssen pharmaceutical. In the group of 18 to 59 years of age, a total of 62% reported having experienced one or more adverse effects, being the mainly fatigue (43,8%), headache (44,4%), myalgia (39,1%), nausea (15,5%) and fever (12,8%). In people older than 60, 35% experienced some adverse effects, such as fatigue (29,7%), headache (30,4%), myalgia (24%), nausea (10,8%) and fever (3,1%).

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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

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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 CoronaVac [6 µg per 0·5 mL of aluminium hydroxide diluent per dose]). 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 µg 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.

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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.

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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 μ g and 6 μ g 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 assay¹⁷ with a minimum four-fold dilution, and neutralising antibodies to pseudovirus¹⁸ 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.

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 pseudovirus. 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

See Online for appendix 2

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

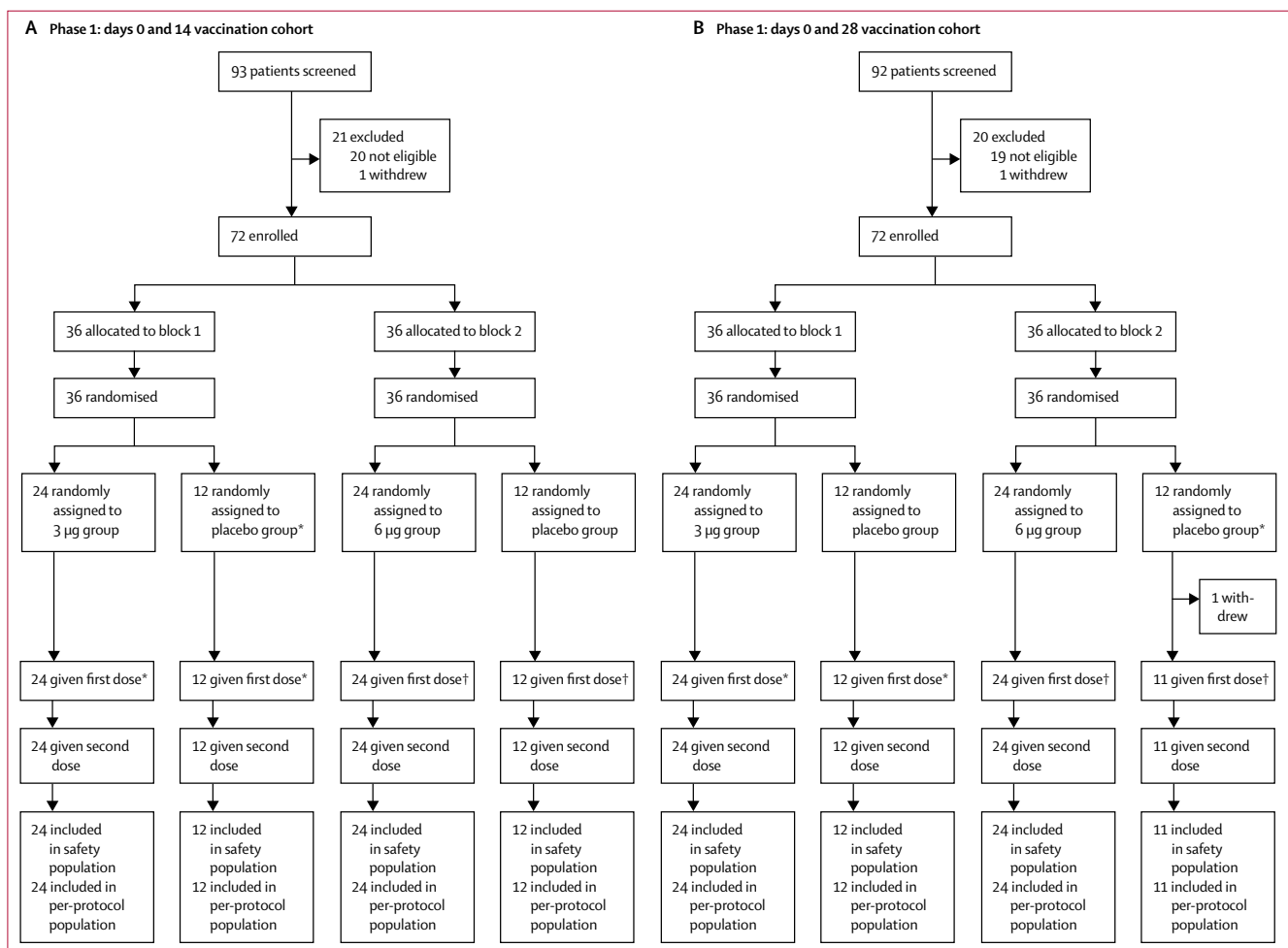
The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All the authors have full access to all the data in the study and the corresponding authors had final responsibility for the decision to submit for publication.

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)

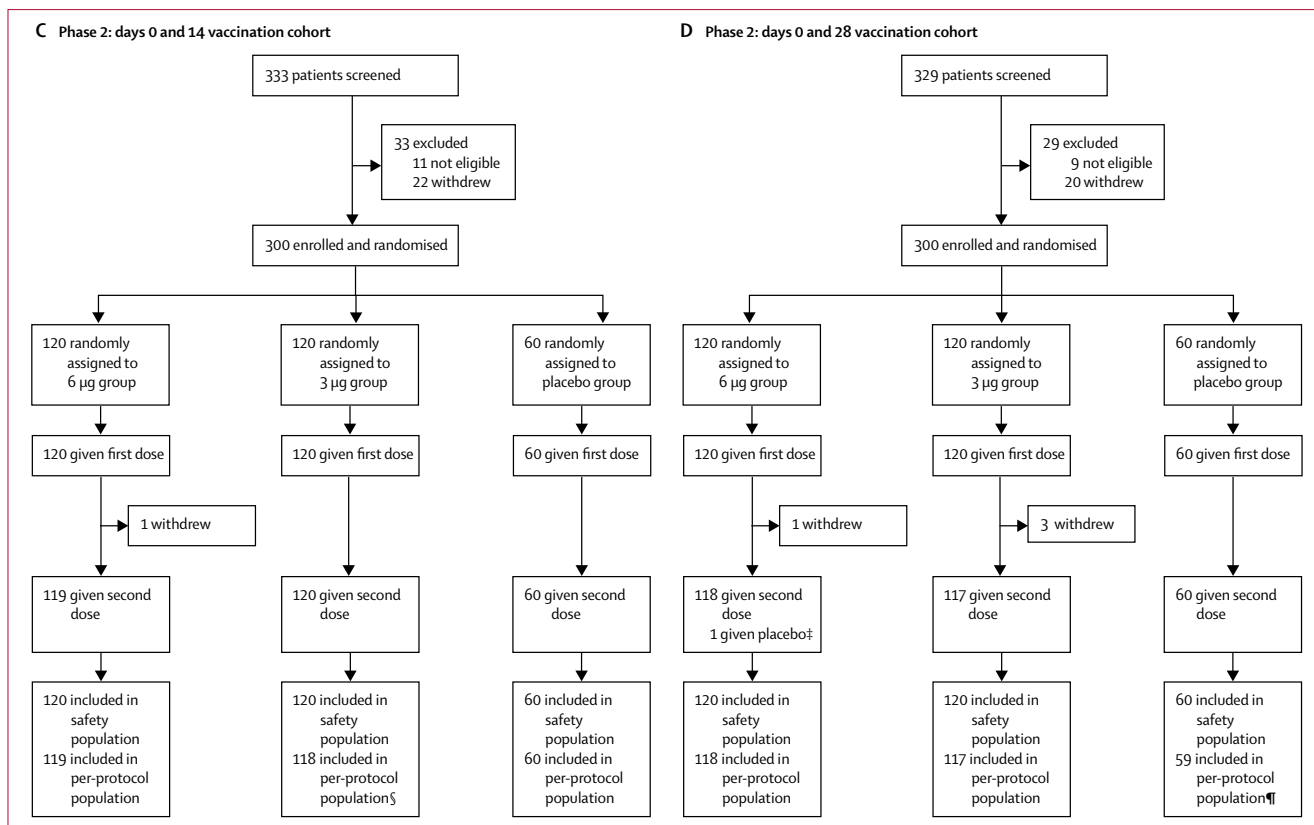


Figure 1: Study profile

*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).

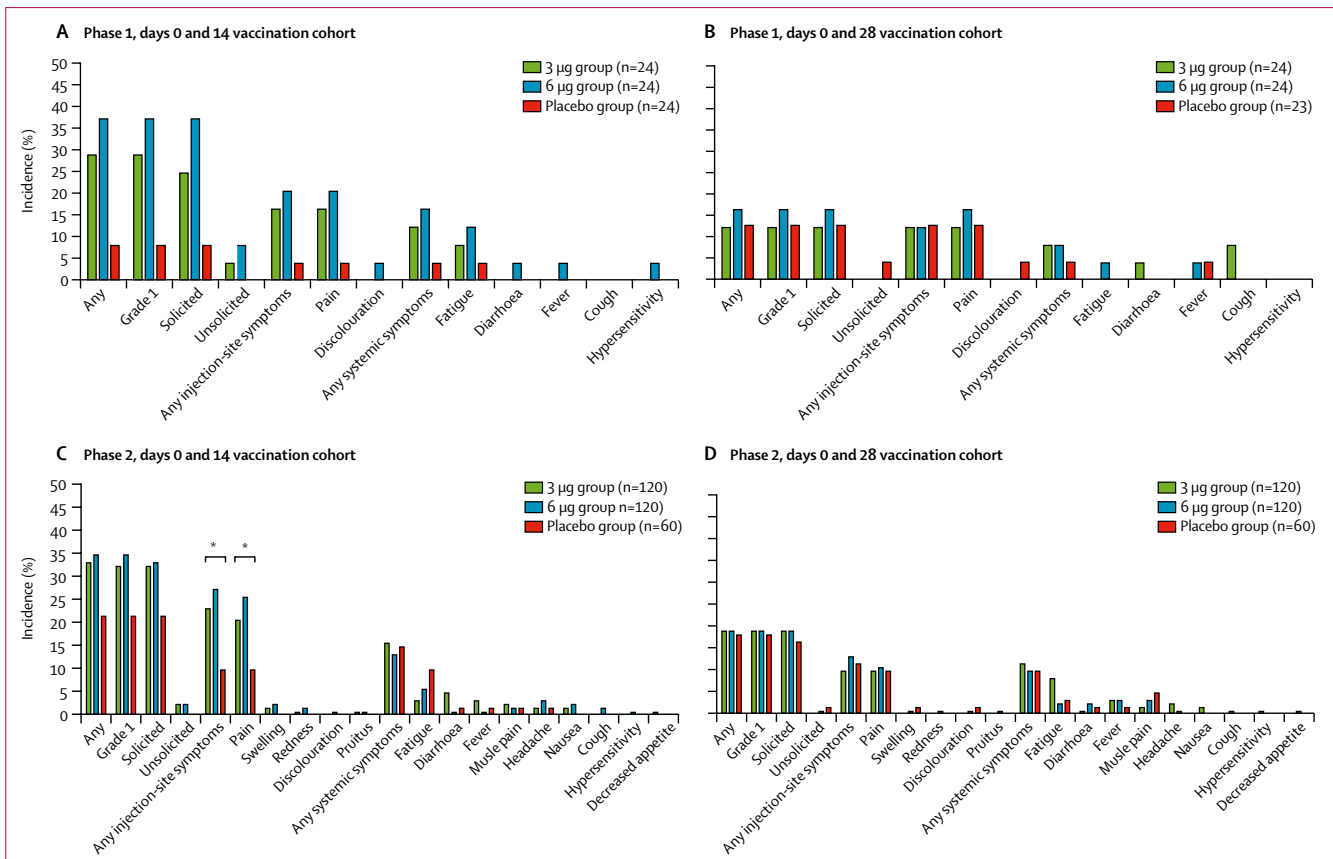


Figure 2: Incidence of adverse reactions reported within 28 days after second dose of study drug, in the days 0 and 14 vaccination cohort in phase 1 (A) and phase 2 (C) and in the days 0 and 28 vaccination cohort in phase 1 (B) and phase 2 (D)
 Adverse reactions refer to the adverse events related to 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

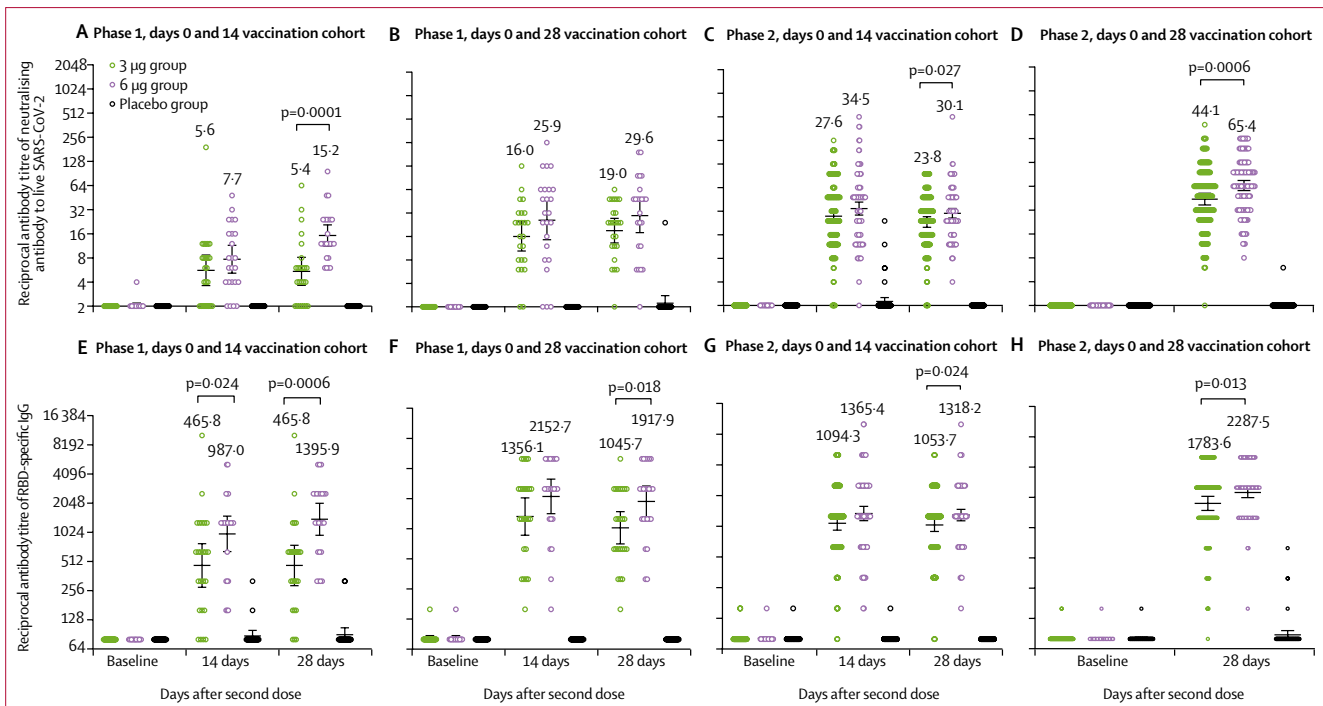


Figure 3: Antibody titres of neutralising antibodies to live SARS-CoV-2 (A–D) and RBD-specific IgG (E–H) induced after two doses of CoronaVac or placebo given in the days 0 and 14 and days 0 and 28 vaccination cohorts, in the phase 1 and phase 2 trials

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 study¹⁵ 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 supported 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.

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JAMA Insights

Reactogenicity Following Receipt of mRNA-Based COVID-19 Vaccines

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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).

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; 1920 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 com-

pared 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

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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.

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Additional Information: See eg, 45 CFR part 46.102(f)(2); 21 CFR part 56; 42 USC §241(d); 5 USC §552a; 44 USC §3501 et seq.

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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

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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 (2.2×10^{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 ($3.5\text{--}6.5 \times 10^{10}$ 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 (MNA₅₀). 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

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(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_{50} 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.

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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 glycoprotein¹⁵ 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 and 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 $2 \cdot 2 \times 10^{10}$ virus particles and a standard dose of $3 \cdot 5\text{--}6 \cdot 5 \times 10^{10}$ 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 vialled by Advent (Pomezia, Italy), and one manufactured by COBRA Biologics (Keele, UK) and vialled 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 (2.2×10^{10} virus particles) or the standard dose ($3.5\text{--}6.5 \times 10^{10}$ 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 trial¹⁸ 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

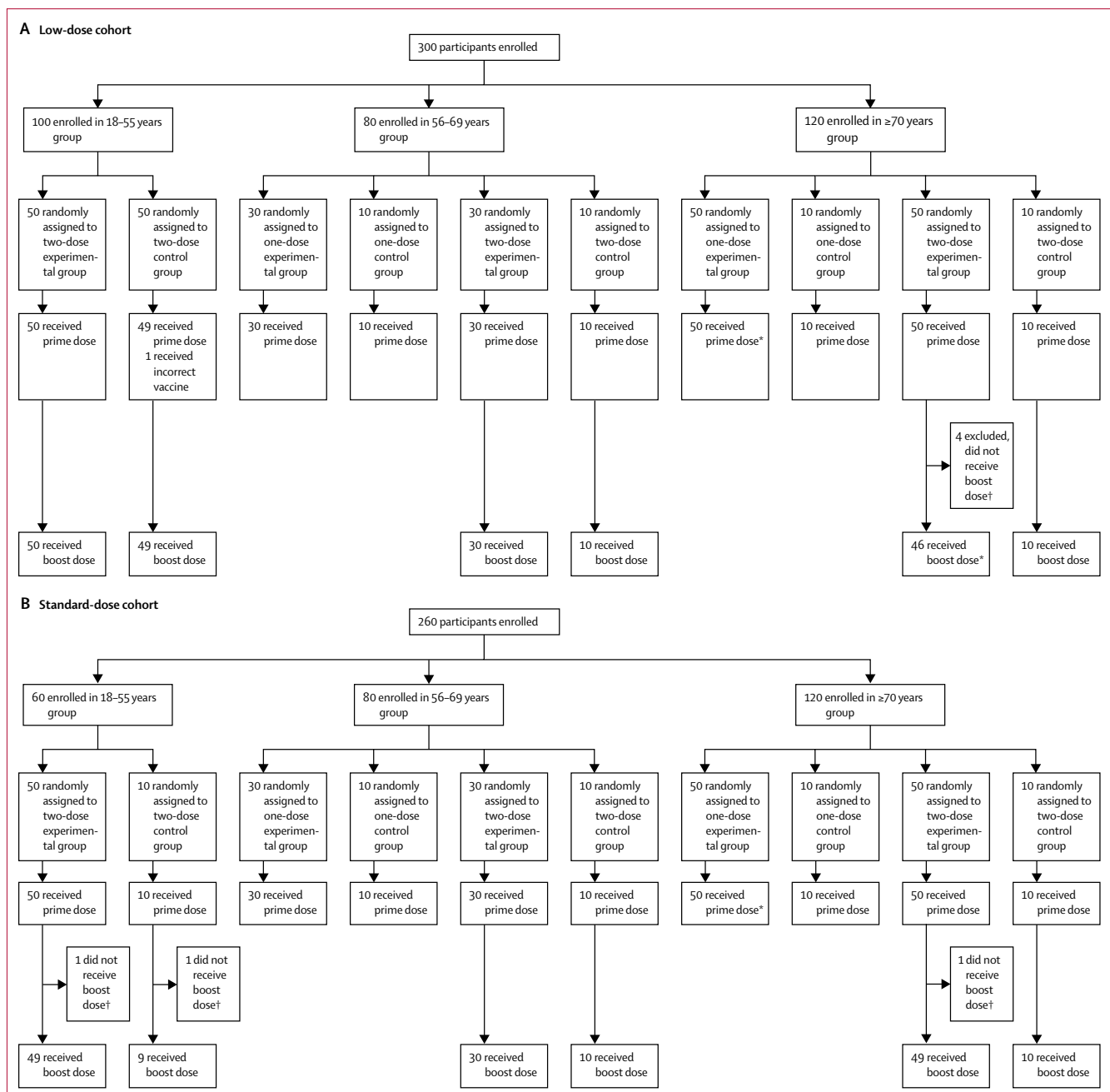


Figure 1: Study profile for the low-dose (A) and standard-dose (B) cohorts

*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, one dose	MenACWY, one dose	ChAdOx1 nCoV-19, two doses	MenACWY, two doses
	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/m ² , 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/m ² , 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

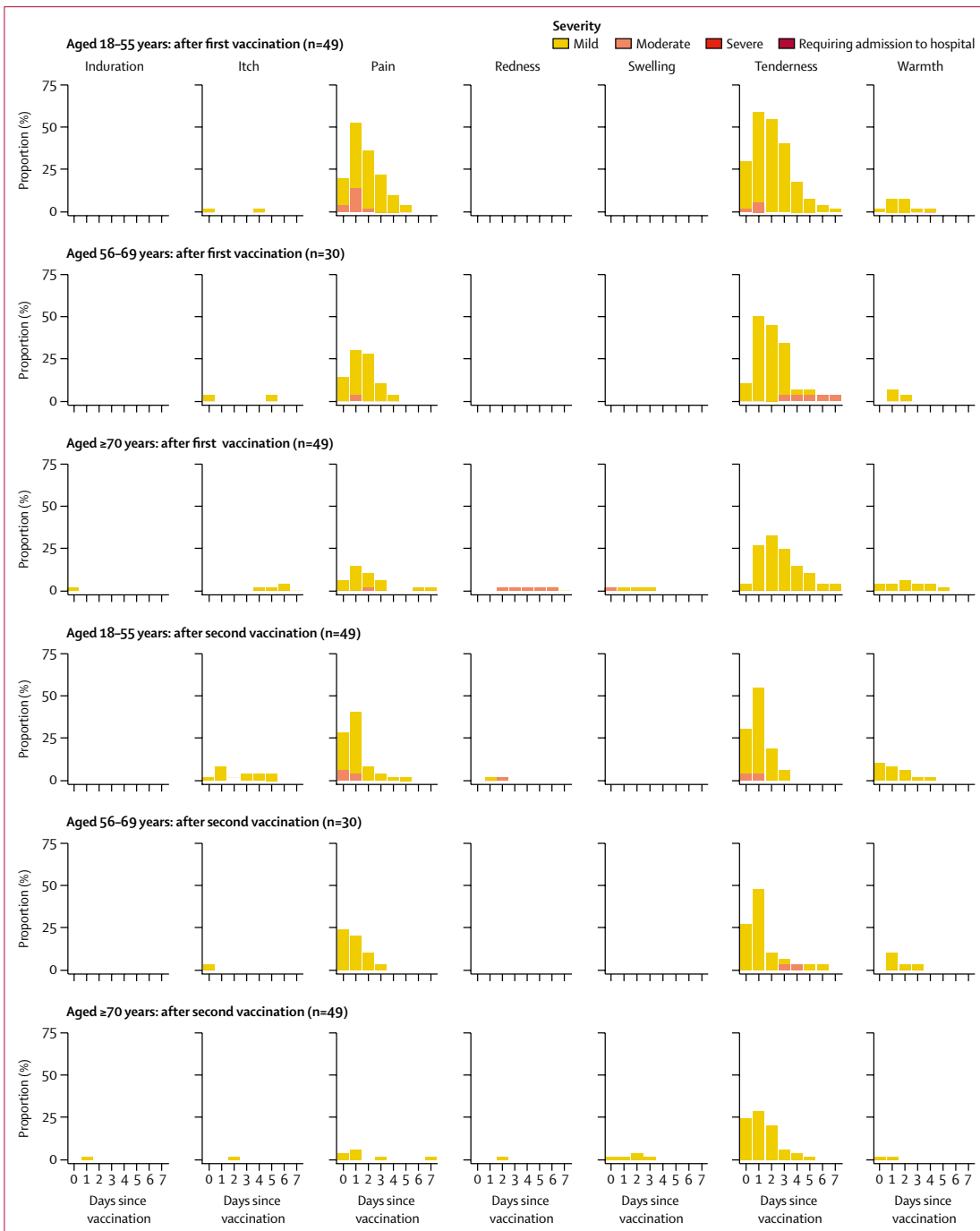


Figure 2: Solicited local adverse reactions in the 7 days after prime and boost doses of standard-dose vaccine, by age. Day 0 is the day of vaccination. Participants shown are those randomly assigned to receive two doses, and data are only shown for participants who received both doses of vaccine.

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

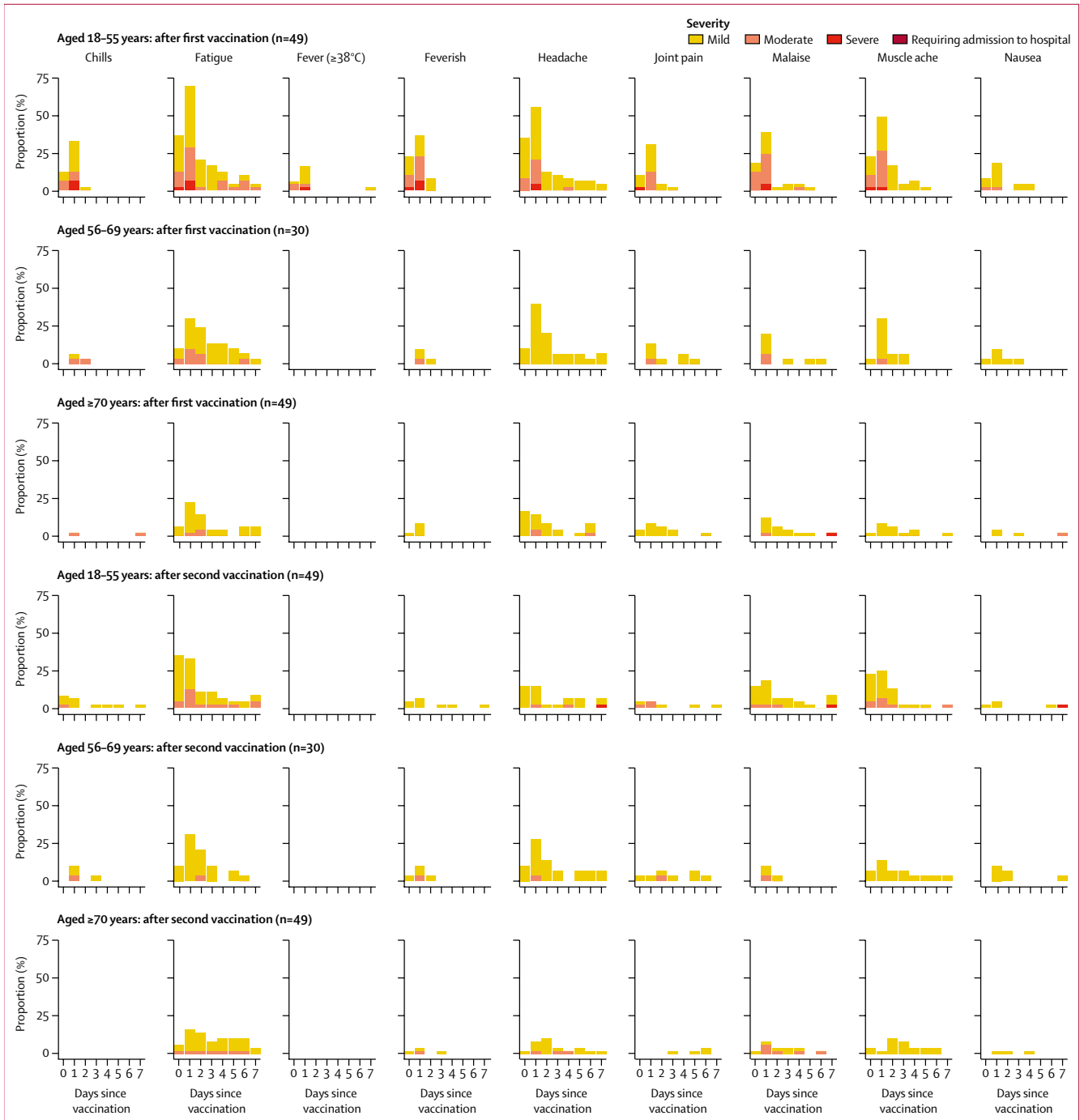


Figure 3: Solicited systemic adverse reactions in the 7 days after prime and boost doses of standard-dose vaccine, by age. Day 0 is the day of vaccination. Feverish is self-reported feeling of feverishness, whereas fever is an objective fever measurement (mild: 38.0 to <38.5°C, moderate: 38.5 to <39.0°C, 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 20713 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_{50}), 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.

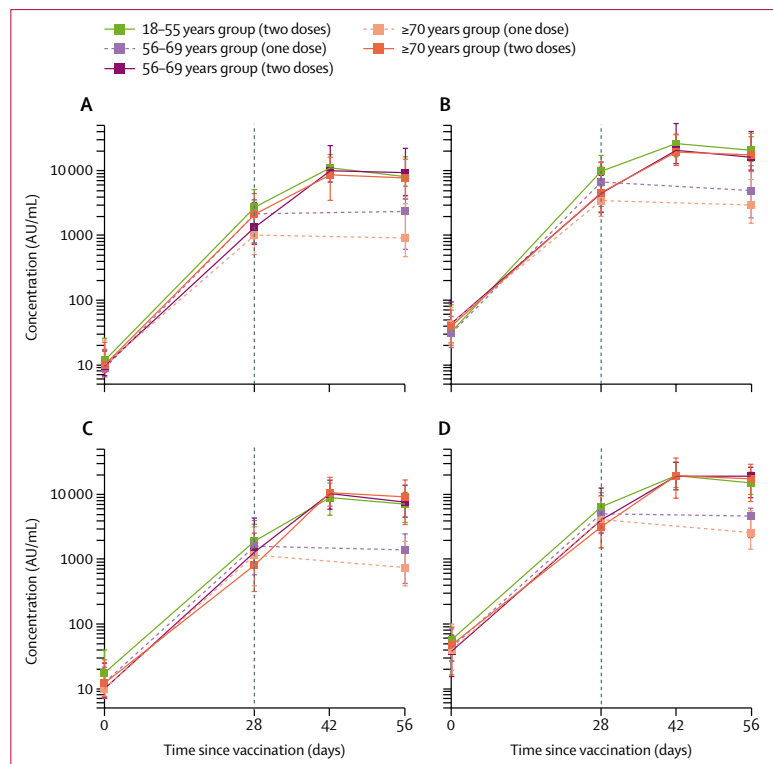


Figure 4: SARS-CoV-2 IgG response to the receptor binding domain in the standard-dose groups (A) and low-dose groups (C) and the spike protein in the standard-dose groups (B) and the low-dose groups (D), by age

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

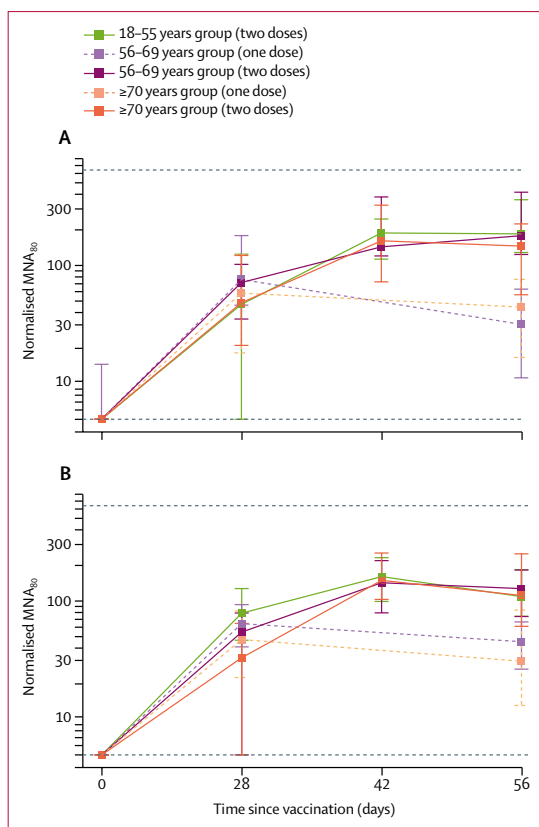


Figure 5: Neutralising antibody titres measured using a live SARS-CoV-2 microneutralisation assay (MNA₉₀) after prime and boost doses of vaccine in standard-dose groups (A) and low-dose groups (B), by age group and vaccine dose
Datapoints are medians, with whiskers showing the IQR. Solid lines show participants 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 log₁₀ ratios. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

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

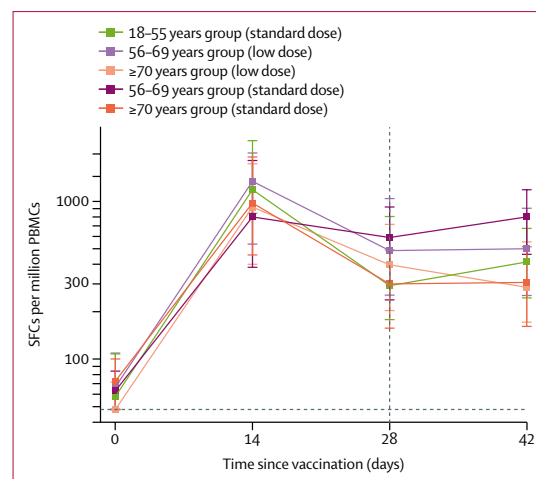


Figure 6: IFN-γ ELISpot response to peptides spanning the SARS-CoV-2 spike insert after prime and boost doses of vaccine for all participants who were given two doses of vaccine, by age group and vaccine dose
ELISpot data were unavailable for the 18–55 years low-dose group because PBMCs were not collected 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.

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 vaccine¹⁸ 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 infection⁸ 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

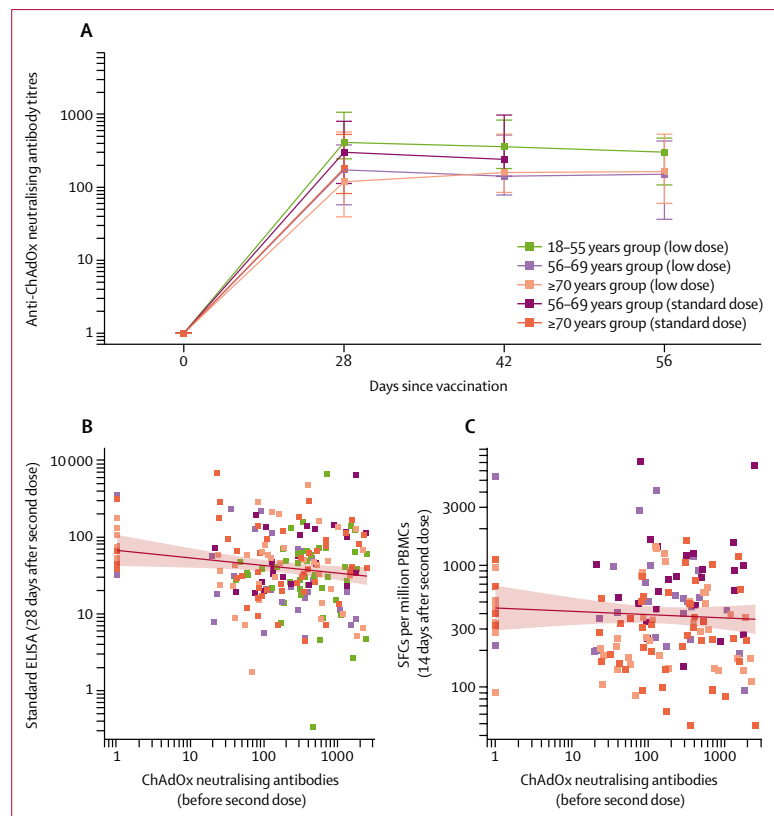


Figure 7: Anti-ChAdOx1 vector neutralising titres after prime and boost doses of vaccine, by age and vaccine dose, and the correlation between pre-boost dose anti-ChAdOx1 neutralising antibodies and 28 days after boost dose antibody and T-cell responses

(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 group¹¹ (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) vaccine²⁹ 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.

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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: $\geq 39.0 - \leq 40.0^{\circ}\text{C}$ or $\geq 102.1 - \leq 104.0^{\circ}\text{F}$

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.

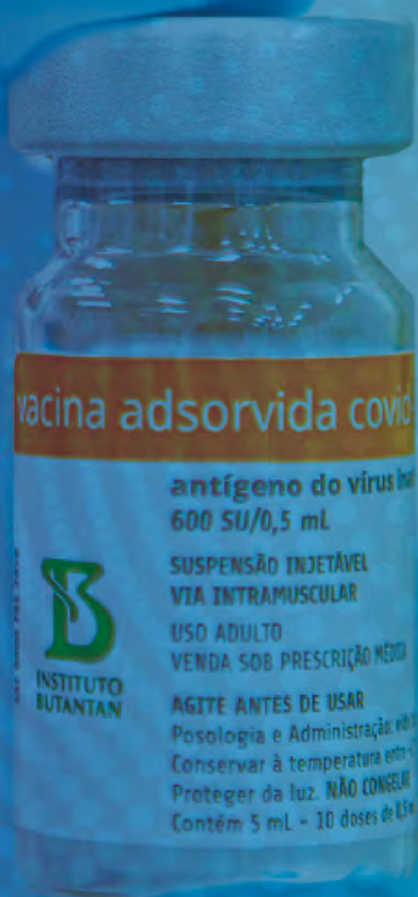
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